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THE HEALTH EFFECTS OF AROMATIC AMINES – A REVIEW

Technical Report

Prepared by

MONITORING AND ASSESSMENT RESEARCH CENTRE
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INTRODUCTION

This review discusses the current state of knowledge regarding the potential human health hazard following exposure to aromatic amines. The aromatic amines (arylamines) are a class of chemicals derived from aromatic hydrocarbons, e.g. benzene, naphthalene, diphenyl, etc., by the replacement of at least one ring hydrogen atom by an amino ($-NH_2$) group. This document does not review in detail compounds related to aromatic amines such as aromatic amides, nitrosamines, carbamates, ureas, hydrazines or azobenzenes (except aminoazobenzenes), although such compounds have been cited where relevant. Similarly, the human health effects of drugs and naturally occurring products have not been addressed specifically.

The chemicals included are subdivided for the most part into five groups (see Appendix, Figure 1) based on chemical structure: (a) anilines (defined for the purpose of this report as monocyclic aromatic amines with one amino group) and phenylenediamines; (b) benzidines (including benzidine- and benzidine-congener-based dyes. Reference in the text to benzidine-based dyes or pigments indicates those that are based on the parent compound benzidine. Substituted benzidine-based compounds are referred to specifically or as benzidine-congener-based compounds); (c) 4,4'-diaminodiphenylmethanes; (d) naphthylamines and (e) aminoazobenzenes.

Within each group specific compounds have been selected for a more detailed discussion of properties and effects. The compounds reviewed were selected according to their past or present technical and/or commercial importance, and the amount of relevant information available. There may, however, have been a reduction or discontinuation of manufacture and/or use of some of these chemicals in certain countries. Structures for selected compounds are illustrated in the Appendix (Figure 2) and are referred to in the text and tables by compound numbers (in brackets).

The review is divided into two parts: Part I deals with environmental and occupational exposure and contains information on production, uses, sources and exposure levels. Part II deals with toxicity and contains information on metabolism and effects seen in humans (where available) together with relevant supplementary information from animal studies and short-term tests. A section has also been included on comparative metabolism and structure-activity relationships in order to provide an insight into the biochemical aspects of aromatic amine toxicity.

The health effects of aromatic amines —A review

by L. K. Shuker,* S. Batt, I. Rystedt** and M. Berlin

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Present address:
*International Agency for Research on Cancer, 150 cours Albert-Thomas, 69372 Lyon, France

**Department of Occupational Dermatology, National Board of Occupational Safety and Health and Karolinska Hospital, S-17184 Solna, Sweden

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Preface

A vast array of literature on aromatic amines has accumulated since the first report by Rehn in 1895 on the increased incidence of bladder cancer in "aniline-dye" workers. Since that time exposure to aromatic amines has been shown to cause a variety of pathological effects in humans. These effects may be highly variable from one compound to another.

In view of the known inherent toxicity and extensive commercial importance of many aromatic amines, the International Programme on Chemical Safety (IPCS)* has supported a literature review and preliminary assessment of their hazards to human health.

It was recognized that the most important data with respect to the assessment of human health risks are first, the degree of toxicity; second, the extent and degree of exposure; and third, the relationship between exposure levels and toxic response. Because of the large amount of information on animal toxicity it was decided that only data directly elaborating those effects seen in humans should be included. The strategy of this study was therefore to review the relevant literature with an emphasis on the above criteria in order to highlight those compounds or groups of compounds requiring further detailed evaluation and research.

*IPCS is a joint UNEP/ILO/WHO activity.

Contents

p-anisidine (13), by the catalytic hydrogenation of 4-nitroanisole, or the Bechamp reduction (with iron filings and HCl) (IARC, 1982a);

p-cresidine (15), by the methylation of 2-nitro-*p*-cresol to the corresponding methyl ether and subsequent reduction of the nitro group (IARC, 1982a);

anthranilic acid (16), via the Hofmann reaction by reaction of phthalimide with sodium hypochlorite and caustic soda (IARC, 1978);

p-aminobenzoic acid (17), by catalytic hydrogenation of 4-nitrobenzoic acid using a platinum or palladium catalyst or by reduction with tin or iron and HCl (IARC, 1978);

4-amino-2-nitrophenol (24), by (1) heating 3-nitrophenylhydroxylamine with sulphuric acid, (2) nitrating *N*-acetyl-4-aminophenol followed by hydrolysis with dilute H_2SO_4, (3) reducing 4'-hydroxy-3'-nitroazobenzene-4-sulphonic acid (IARC, 1978);

5-nitro-*o*-anisidine (25), by (1) the nitration of *o*-anisidine, (2) the partial reduction of 2,4-dinitroanisole (IARC, 1982a);

p-chloro-*o*-toluidine (30), by (1) reduction of 2-nitrotoluene with tin and HCl followed by chlorination, (2) using chlorination of *o*-toluidine, (3) chlorination of acetotoluidine and saponification to the free amine (IARC, 1978);

m-phenylenediamine (32), by the reduction of 1,3-dinitrobenzene with (1) iron and HCl or (2) iron, ammonium polysulphide and water gas (IARC, 1978);

p-phenylenediamine (33), by the reduction of 1-amino-4-nitrobenzene with (1) iron and HCl, or (2) iron, ammonium polysulphide and hydrogen, or (3) iron and $FeCl_2$ (IARC, 1978);

2,4-diaminotoluene (34), by the nitration of toluene followed by catalytic reduction to a mixture of diamines (2,4-isomer (80%) and 2,6-isomer (20%)) (IARC, 1978);

2,5-diaminotoluene (35), by the electrolytic reduction of 2,5-dinitrotoluene or reductive cleavage of *o*-aminoazotoluene (70) with zinc dust and HCl (IARC, 1978);

2,4-diaminoanisole (36), by the methoxylation of 2,4-dinitro-l-chlorobenzene followed by reduction with iron (IARC, 1978; 1982a);

1,2-diamino-4-nitrobenzene (40), by reduction of 2,4-dinitroaniline with alcoholic ammonium sulphide (IARC, 1978);

1,4-diamino-2-nitrobenzene (41), by acetylation of *p*-phenylenediamine with acetic anhydride followed by nitration and hydrolysis (IARC, 1978);

4-chloro-*o*-phenylenediamine (42), by the reduction of 4-chloro-1,2-dinitrobenzene (IARC, 1982a);

4-chloro-*m*-phenylenediamine (43), by the reduction of 4-chloro-1,3-dinitrobenzene with iron, or $ZnCl_2$ and HCl (IARC, 1982a).

No information was found on the commercial production of *m*-cresidine (14), and no evidence for production of commercial quantities of 2,4,5-(10) and 2,4,6-(11) trimethylaniline in the U.S.A., Western Europe or Japan (IARC, 1982a).

1.2 Benzidines

Commercial production of benzidine (45) involves the alkaline reduction of nitrobenzene to hydrazobenzene. Hydrazobenzene is separated from the reaction mass (containing azobenzene (64) and azoxybenzene) and rearranged to benzidine by

Part I EXPOSURE

Sections 1–4 review information relating to the source, type and extent of exposure to aromatic amines to which both the general and working populations may be subjected. In order to evaluate fully the significance of possible exposure to a given compound, such information must be taken into account. The chemicals are discussed according to the subdivisions outlined in the introduction (see also Figure 1 in the Appendix).

1. COMMERCIAL PRODUCTION

Exposure to aromatic amines may occur during both production and use. A review of some commercial production methods has been made and, when they are known, likely impurities or contaminants in the final product have been indicated since these also may be implicated in any toxicity associated with the final product.

1.1 Anilines and phenylenediamines

Many substituted anilines are prepared by reduction of the corresponding nitro-compound, by methods which include catalytic hydrogenation or chemical reduction, for example, with iron/HCl. Some commercial production methods include:

aniline (1) by (1) the catalytic hydrogenation of nitrobenzene, (2) the amination of chlorobenzene (only method used in U.S.A.) and (3) amination of phenol (Japan) (IARC, 1982a);

o-toluidine (2), by reduction of 1-methyl-2-nitrobenzene (iron or catalytic hydrogenation) (IARC, 1982a);

2,4-xylidine (5), by nitration and reduction of xylene and formation of the acetate salt for separation of the isomeric mixture (i.e. from 2,5-xylidine) (IARC, 1978); and

2,5-xylidine (6), as for 2,4-xylidine and then precipitation with hydrochloric acid (IARC, 1978).

o-anisidine (12), by the catalytic hydrogenation of 2-nitroanisole, or the Bechamp reduction (with iron filings and HCl) (IARC, 1982a);

treatment with HCl (Ferber, 1978). Contaminants in the final product include diphenyline and aniline (1) (Haley, 1975).

The synthesis of benzidine congeners is similar to benzidine synthesis, with nitrobenzene congeners being used as the starting material. The syntheses of some benzidine congeners of significant industrial/commercial value together with contaminants are shown in Table 1.

1.2.1 Benzidine-based dyes and pigments

Production of dyes and pigments from benzidine (45) and its congeners proceeds via tetrazotization to form the tetrazonium salt, followed by coupling with suitable secondary components (e.g. hydroxylated aromatics or arylamines) to form coloured products (EPA, 1979).

Benzidine and other compounds such as 4-aminobiphenyl (72), 2,4-diaminoazobenzene (chrysoidine) (66), diphenyline and semidine may be present in the final dye product both as a result of impurities introduced in manufacturing processes or as decomposition products. If these substances do not interfere with the dyeing process, ordinarily they are not removed (NIOSH, 1980). Concentrations of benzidine ranging from less than 1 – 224 ppm have been reported in samples of benzidine-based dyes from eight countries. In one commercially produced sample of Direct Black 38 (76) (a benzidine-based dye), 150 ppm of 4-aminobiphenyl, 9200 ppm of 2,4-diaminoazobenzene and 0.1 ppm benzidine were detected (EPA, 1979).

1.3 4,4′-Diaminodiphenylmethanes

The commercial production of the 4,4′-diaminodiphenylmethanes considered herein is from the condensation of the related parent aniline with formaldehyde (IARC, 1974, 1982a). In the case of the parent compound, 4,4′-methylenedianiline (53), condensation of aniline (1) with formaldehyde under acidic conditions yields a mixture of di-, tri- and polyamines, from which the diamine can be isolated (Moore, 1978). Only relatively small amounts of 4,4′-methylenedianiline are isolated in the pure form. The primary use for the unpurified mixture is the production of a di-, tri- and polyisocyanate mix by reaction with phosgene. The mixed isocyanate is sold commercially as polymethylenepolyphenylisocyanate. The diisocyanate, 4,4′-methylenediphenylisocyanate (MDI), may be separated from the mixed isocyanate and refined (IARC, 1974). The uses of MDI are dependent on the relative amounts of di-, tri- and polyamine present in the mix prior to reaction with phosgene.

Commercial 4,4′-methylenebis (2-chloroaniline) (54) may contain up to 10% polyamines and about 0.9% *o*-chloroaniline (26) as a residue from the manufacturing process. *o*-Chloroaniline may also be released as a decomposition product on heating above 200°C (HSE, 1983).

1.4 Naphthylamines

1-Naphthylamine (57), 2-naphthylamine (58) and *N*-phenyl-2-naphthylamine (60) have all been produced commercially for more than 50 years (IARC, 1974, 1978).

Table 1 The synthesis of selected benzidine congeners

	Starting material	Intermediate	Product	Contaminants in the final product
1	*o*-nitrotoluene	hydrazotoluene	3,3′-dimethylbenzidine (46)	ditolyline and *o*-toluidine (2)
2	*m*-nitrotoluene	hydrazotoluene	2,2′-dimethylbenzidine (47)	ditolyline and *m*-toluidine (3)
3	*o*-nitrochlorobenzene	hydrazochlorobenzene	3,3′-dichlorobenzidine (50)	*o*-chloroaniline (26)
4	2,5-dichloronitrobenzene	tetrachloro-substituted hydrazobenzene	2,2′,5,5′-tetrachlorobenzidine (52)	2,5-dichloroaniline
5	*o*-nitroanisole	hydrazoanisidine	3,3′-dimethoxybenzidine (48)	*o*-anisidine (12)

Adapted from Haley (1975)

1-Naphthylamine is probably manufactured by the catalytic hydrogenation of 1-nitronaphthalene using a nickel catalyst. 2-Naphthylamine may be prepared by the reaction of 2-naphthol with ammonia and ammonium sulphite, though its production has been discontinued in several countries including the U.K. prior to 1960 (IARC, 1974) and in the U.S.A. in 1972 (Fishbein, 1980). *N*-phenyl-2-naphthylamine is made by the condensation of 2-naphthol with aniline (1) in the presence of an acid catalyst (Dressler, 1978). However, it is probable that its production is also limited at present and, for example, it is thought no longer to be produced in the U.S.A. (Fishbein, 1980). 1,5-Naphthalenediamine (61) and 1,8-naphthalenediamine (62) may both be produced from metal/acid reduction or catalytic hydrogenation of the related dinitronaphthalene (Dressler, 1978). 1,5-Naphthalenediamine may also be prepared by ammonolysis of 1,5-dihydroxynaphthalene.

Commercial 1-naphthylamine and *N*-phenyl-2-naphthylamine may both be contaminated by 2-naphthylamine. Recent production methods for 1-naphthylamine have been reported to contain up to 0.5% 2-naphthylamine. However, in the past levels may have been as high as 4−10%. 2-Naphthol also has been reported as a contaminant of *N*-phenyl-2-naphthylamine. 2-Naphthylamine itself has been reported to have been contaminated with polyaromatic heterocyclic compounds such as 3,4,5,6-dibenzophenazine, formed from 2-naphthylamine in the presence of air, and 2-amino-1,4-naphthoquinone-N^4,2-naphthylimine (IARC, 1974, 1978).

1.5 Aminoazobenzenes

The methods used for commercial production were not found in the literature for any of the aminoazobenzenes described herein; however, *p*-aminoazobenzene (65) may be prepared by the diazotization of aniline (1) and coupling of the resultant diaminoazobenzene with a mix of aniline and aniline hydrochloride; *o*-aminoazotoluene (70) may be synthesized by the diazotization of *o*-toluidine (2); diacetylaminoazotoluene (71) may be prepared by acetylation of aminoazotoluene with acetic anhydride or acetylchloride in the presence of sodium acetate; and *p*-dimethylaminoazobenzene (67) may be prepared by sodium nitrite treatment of an aniline/dimethylaniline mix in sodium hydroxide (IARC, 1975).

1.6 Production and import volumes

Some available production and import volumes for several aromatic amines are exemplified in Table 2. Several hundreds of millions of kilogrammes of aniline are produced annually. Yearly production figures for *o*-toluidine (2), 2,4-xylidine (5), *o*-anisidine (12) and *p*-anisidine (13), 2,4-diaminotoluene (34), 3,3′-dichlorobenzidine (50) and 4,4′-methylenedianiline (53) are also of the order of millions of kilogrammes and, until the early to mid-1970s, 4,4′-methylenebis(2-chloroaniline) (54), 1-naphthylamine (57) and *N*-phenyl-2-naphthylamine (60) were produced in similar quantities. No recent reports of 2-naphthylamine (58) production have been found and benzidine (45) only appears to have been produced in small quantities (thousands of kilogrammes annually) in recent years. Sales and import data (U.S.A.) for commercially important dyes and pigments based on benzidine and congeners are

Table 2 Production and import volumes for aromatic amines

Compound	Compound No.	Country	(year)	Production volume/capacity 10^3 kg/y	Imports 10^3 kg/y	Reference
Anilines						
aniline	(1)	Belgium	(1983)	102,000		SRI (1983a)
		France	(1983)	25,000		SRI (1983a)
		F.R.G.	(1983)	200,000		SRI (1983a)
		Japan	(1979)	79,000		IARC (1982a)
		Portugal	(1983)	50,000		SRI (1983a)
		Spain	(1983)	5,000		SRI (1983a)
		Switzerland	(1983)	12,000		SRI (1983a)
		U.K.	(1983)	115,000		SRI (1983a)
		U.S.A.	(1978)	275,000		IARC (1982a)
		U.S.A.	(1979)	313,000	19.7	IARC (1982a)
		U.S.A.	(1983)	642,000 (1,412 10^6 lb)		SRI (1983b)
o-toluidine	(2)	Japan	(1979)	1,800	88	IARC (1982a)
		U.S.A.	(1976)		11 (25 10^3 lb)	NTP (1983)
		U.S.A.	(1977)	>450–4,540		IARC (1982a)
		U.S.A.	(1979)		1,370	IARC (1982a)
2,4-xylidine	(5)	F.R.G.		100–1,000		IARC (1978)
		Japan	(1975)	20–30		IARC (1978)
		U.S.A.	(1974)		64.3	IARC (1978)
2,5-xylidine	(6)	U.K.			10–100	IARC (1978)
		U.S.A.	(1974)		3.7	IARC (1978)

Table 2/contd

Compound	Compound No.	Country	(year)	Production volume/capacity 10^3 kg/y	Imports 10^3 kg/y	Reference
Anilines contd						
o-anisidine	(12)	Japan	(1974)		1,000	IARC (1982a)
		Japan	(1979)		669	IARC (1982a)
		U.S.A.	(1979)	>2.3 (>5 10^3 lb)[a]	1,260	IARC (1982a) NTP (1983)
		U.S.A.	(1980)		1,100 (2.4 10^6 lb)	NTP (1983)
p-anisidine	(13)	Japan	(1979)	1,140		IARC (1982a)
		U.S.A	(1979)		183	IARC (1982a)
m-cresidine	(14)	U.S.A.	(1976)		0.6	IARC (1982a)
p-cresidine	(15)	Japan	(1979)		>500	IARC (1982a)
		U.S.A.	(1979)		267	IARC (1982a)
anthranilic acid	(16)	Japan	(1973)	57	nil	IARC (1978)
		Japan	(1974)	88	nil	IARC (1978)
		Japan	(1975)	44	nil	IARC (1978)
		Switzerland			10–100	IARC (1978)
		U.K.		10–100		IARC (1978)
		U.S.A.	(1972)		102	IARC (1978)
		U.S.A.	(1973)		7	IARC (1978)
		U.S.A.	(1975)		105	IARC (1978)
		U.S.A.	(1976)		5	IARC (1978)

Table 2/contd

Compound	Compound No.	Country	(year)	Production volume/capacity 10^3 kg/y	Imports 10^3 kg/y	Reference
Anilines contd						
4-amino-2-nitrophenol	(24)	Japan		<0.1		IARC (1978)
		Switzerland			10–100	IARC (1978)
		U.S.A.	(1972)		84.4	IARC (1978)
		U.S.A.	(1973)		81.2	IARC (1978)
		U.S.A.	(1974)		55.7	IARC (1978)
		U.S.A.	(1975)		53.4	IARC (1978)
5-nitro-*o*-anisidine	(25)	Japan	(1979)	>400		IARC (1982a)
		U.S.A.	(1977)	4.5–45.4		IARC (1982a)
		U.S.A.	(1979)	16 (37 10^3 lb)	13	IARC (1982a) NTP (1983)
		U.S.A.	(1980)	>6.8 (>15 10^3 lb)	10 (23 10^3 lb)	NTP (1983)
p-chloro-*o*-toluidine	(30)	Japan	(1973)		14	IARC (1978)
		Japan	(1974)		3	IARC (1978)
		Japan	(1975)		4	IARC (1978)
		Switzerland	(1976)	100–200		IARC (1978)
		U.S.A.	(1973)		86.3	IARC (1978)
		U.S.A.	(1974)		73.3	IARC (1978)
		U.S.A.	(1975)		2.4	IARC (1978)
Phenylenediamines						
m-phenylenediamine	(32)	Japan	(1974)	296		IARC (1978)
		Japan	(1975)	171		IARC (1978)
		Switzerland			100–1,000	IARC (1978)
		U.K.		10–100	<50	IARC (1978)
		U.S.A.	(1972)		146.7	IARC (1978)

Table 2/contd

Compound	Compound No.	Country	(year)	Production volume/capacity 10^3 kg/y	Imports 10^3 kg/y	Reference
Phenylenediamines contd						
		U.S.A.	(1974)		90.1	IARC (1978)
		U.S.A.	(1975)		35.2	IARC (1978)
p-phenylenediamine	(33)	Japan	(1971)	80		IARC (1978)
		Japan	(1972)	60		IARC (1978)
		Japan	(1973)	114		IARC (1978)
		Japan	(1974)	113		IARC (1978)
		Japan	(1975)	55		IARC (1978)
		Switzerland			10 - 100	IARC (1978)
		U.K.		10 – 100	<100	IARC (1978)
		U.S.A.	(1972)		75.3	IARC (1978)
		U.S.A.	(1973)		12.0	IARC (1978)
		U.S.A.	(1974)		29.2	IARC (1978)
2,4-diaminotoluene	(34)	U.S.A.	(1974)	233,600[d]	35	IARC (1978)
		U.S.A	(1975)	217,400[d]	20	IARC (1978)
		U.S.A.	(1980)	98,200 (216 10^6 lb)	1.8 (4 10^3 lb)	NTP (1983)
2,5-diaminotoluene	(35)	Japan	(1976)	850[b]		IARC (1978)
		U.S.A.	(1974)		1.3	IARC (1978)
		U.S.A.	(1974)		29.4[c]	IARC (1978)
		U.S.A.	(1975)		3.4	IARC (1978)
2,4-diaminoanisole	(36)	Japan	(1975)	7	nil	IARC (1978)
		Switzerland			10 – 100	IARC (1978)
		U.S.A.	(1974)		16	IARC (1978)
		U.S.A.	(1975)		5	IARC (1978)
		U.S.A.	(1979)		0.16[c] (350 lb)	NTP (1983)

Table 2/contd

Compound	Compound No.	Country	(year)	Production volume/capacity 10^3 kg/y	Imports 10^3 kg/y	Reference
Phenylenediamines contd						
1,2-diamino-4-nitro-benzene	(40)	U.S.A.	(1973)		1.9	IARC (1978)
		U.S.A.	(1975)		1.1	IARC (1978)
1,4-diamino-2-nitro-benzene	(41)	Japan	(1975)		0.1–0.2	IARC (1978)
		U.S.A.	(1974)		1.4	IARC (1978)
		U.S.A.	(1975)		0.2	IARC (1978)
4-chloro-*o*-phenylene-diamine	(42)	U.S.A.	(1977)	0.45–4.5		IARC (1978)
4-chloro-*m*-phenylene-diamine	(43)	U.S.A.	(1970)		4.5	IARC (1978)
Benzidines[c]						
benzidine[f]	(45)	U.S.A.	(1972)	682 (1.5×10^6 lb)		Radding et al. (1977)
		U.S.A.	(1974)	604 (1.33×10^6 lb)		EPA (1979)
		U.S.A.	(1975)	700 (1.54×10^6 lb)		EPA (1979)
		U.S.A.	(1976)	500 (1.1×10^6 lb)		EPA (1979)
		U.S.A.	(1977)	discontinued	4.3 (9.5×10^3 lb)	EPA (1979)
		U.S.A.	(1980)	discontinued	4.1	IARC (1982b)

Table 2/contd

Compound	Compound No.	Country	(year)	Production volume/capacity 10^3 kg/y	Imports 10^3 kg/y	Reference
Benzidines contd						
3,3'-dimethylbenzidine	(46)	U.S.A.	(1971)		44 (98 10^3 lb)	EPA (1979)
		U.S.A.	(1974)	100[b] (220 10^3 lb)	273 (600 10^3 lb)	EPA (1979)
		U.S.A.	(1976)	90 (200 10^3 lb)	215 (473 10^3 lb)	EPA (1979)
		U.S.A.	(1977)	90 (200 10^3 lb)	160 (353 10^3 lb)	EPA (1979)
		U.S.A.	(1978)	90[b] (200 10^3 lb)	150 (331 10^3 lb)	EPA (1979)
		U.S.A.	(1979)		1,590 (3.5 10^6 lb)	NTP (1979)
		U.S.A.	(1980)		>2.3[a] (>5 10^3 lb)	NTP (1983)
3,3'-dimethoxybenzidine	(48)	U.S.A.	(1967)	167 (367 10^3 lb)		EPA (1979)
		U.S.A.	(1972)		124 (273 10^3 lb)	EPA (1979)
		U.S.A.	(1976)		341 (752 10^3 lb)	EPA (1979)
		U.S.A.	(1977)		195 (428 10^3 lb)	EPA (1979)
		U.S.A.	(1978)	1,772 (3.9 10^6 lb)	252 (554 10^3 lb) 39 (86 10^3 lb)	EPA (1979) NTP (1983)
		U.S.A.	(1979)	>4.5[a] (>10 10^3 lb)	35 (77 10^3 lb)	NTP (1983)
		U.S.A.	(1980)		9 (20 10^3 lb)	NTP (1983)
3,3'-dichlorobenzidine	(50)	Japan	(1975)	870		IARC (1982b)
		Japan	(1980)	1,800		IARC (1982b)
		U.S.A.	(1968)	1,300 (2.94 10^6 lb)	422 (929 10^3 lb)	EPA (1979)
		U.S.A.	(1970)	1,700 (3.66 10^6 lb)	445 (980 10^3 lb)	EPA (1979)
		U.S.A.	(1971)		658	IARC (1982b)
		U.S.A.	(1972)	2,900 (6.42 10^6 lb)		EPA (1979)
		U.S.A.	(1975)	1,400[b] (3 10^6 lb)	0	EPA (1979)
		U.S.A.	(1976)	2,000[b] (4.5 10^6 lb)	0.9 (2 10^3 lb)	EPA (1979)
		U.S.A.	(1977)	2,500[b] (5.5 10^6 lb)	0	EPA (1979)

Table 2/contd

Compound	Compound No.	Country	(year)	Production volume/capacity 10^3 kg/y	Imports 10^3 kg/y	Reference
Benzidines contd						
		U.S.A.	(1978)	several million kg[a]	119 (262 10^3 lb)	EPA (1979)
		U.S.A.	(1979)		98.6 (208 10^3 lb)	NTP (1983)
		U.S.A.	(1980)		147	IARC (1982b)
2,2′,5,5′-tetrachloro-benzidine	(52)	U.S.A.	(1978)		0.1	IARC (1982a)
4,4′-Diaminodiphenylmethanes						
isolated methylenedianiline	(53)	U.S.A.	(1965)	>500		IARC (1974)
		U.S.A.	(1966)	>700		IARC (1974)
		U.S.A.	(early 1970s)	1,000[b]		IARC (1974)
		U.S.A.	(1972/73)	900 (900 ton)		Moore (1978)
unisolated methylenedianiline		U.S.A.		81,000 (200 10^6 lb)		Fishbein (1977)
4,4′-methylenebis-(2-chloroaniline)	(54)	U.S.A.	(1970)	1,500–2,500		IARC (1974)
		U.S.A.	(1972)	3,500		IARC (1974)
		U.S.A.	(1977)	168 (369 10^3 lb)		NTP (1983)
		U.S.A.	(1979)	>4.5 (>10 10^3 lb)		NTP (1983)

Table 2/contd

Compound	Compound No.	Country	(year)	Production volume/capacity 10^3 kg/y	Imports 10^3 kg/y	Reference
4,4'-Diaminodiphenylmethanes contd						
4,4'-methylenebis (2-methylaniline)	(55)	U.S.A.	(1974)	<0.45 (<10^3 lb)		Radding et al. (1977)
4,4'-methylenebis (*N*,*N*-dimethylaniline)	(56)	U.S.A.	(1974)	845		IARC (1982a)
		U.S.A.	(1977)	453		IARC (1982a)
		U.S.A.	(1979)	167 (37 10^3 lb)		NTP (1983)
		U.S.A.	(1980)	>4.5 (>10 10^3 lb)		NTP (1983)
purified MDI		U.S.A.	(1970)	5,500[b]		IARC (1974)
		U.S.A.	(1974)	45,700 (45,000 ton)[g]		Moore (1978)
polymeric MDI		Belgium		24,400 (24,000 ton)		Moore (1978)
		France		12,200 (12,000 ton)		Moore (1978)
		F.R.G.		129,000 (127,000 ton)		Moore (1978)
		Italy		30,500 (30,000 ton)		Moore (1978)
		Japan	(1975)	28,900 (28,400 ton)		Moore (1978)
		Netherlands		91,400 (90,000 ton)		Moore (1978)
		Netherlands		61,000 (60,000 ton)		Moore (1978)
		Netherlands		24,400 (24,000 ton)		Moore (1978)
		Spain		45,700 (45,000 ton)		Moore (1978)
		U.K.		40,600 (40,000 ton)		Moore (1978)
		U.S.A.	(late 1960s)	~50,000 (50,000 ton)		Moore (1978)
		U.S.A.	(1970)	52,000[b]		IARC (1974)
		U.S.A.	(1973)	130,000 (128,000 ton)		Moore (1978)

Table 2/contd

Compound	Compound No.	Country	(year)	Production volume/capacity 10^3 kg/y	Imports 10^3 kg/y	Reference
4,4'-Diaminodiphenylmethanes contd						
		U.S.A.	(1974)	173,000 (171,000 ton)		Moore (1978)
		U.S.A.	(1976)	239,800 (236,000 ton)		Moore (1978)
Naphthylamines						
1-naphthylamine	(57)	U.S.A.	(1963)	>500		IARC (1974)
		U.S.A.	(1967)		2	IARC (1974)
		U.S.A.	(1971)		27	IARC (1974)
		U.S.A.	(1972)	3,500 (7.7 10^6 lb)		Radding et al. (1977)
2-naphthylamine	(58)	U.S.A.	(1967)		17.4	IARC (1974)
		U.S.A.	(1974)	<0.45 (<10^3 lb)		Radding et al. (1977)
N-phenyl-2-naphthylamine	(60)	Japan	(1971)	5,060	negligible	IARC (1978)
		Japan	(1974)	2,250	negligible	IARC (1978)
		U.S.A.	(1967)	2,540 (2,500 ton)		Dressler (1978)
		U.S.A.	(1973)	2,240		IARC (1978)
		U.S.A.	(1974)	1,370	53.6	IARC (1978)
		U.S.A.	(1975)	708.7	75.8	IARC (1978)
1,5-naphthalenediamine	(61)	Japan	(1979)	50		IARC (1982a)
		U.S.A.	(1979)		2	IARC (1982a)

Table 2/contd

Compound	Compound No.	Country	(year)	Production volume/capacity 10^3 kg/y	Imports 10^3 kg/y	Reference
Aminoazobenzenes						
azobenzene	(64)	U.S.A.	(1974)	<0.45 (<10^3 lb)		Radding et al. (1977)
p-aminoazobenzene	(65)	U.S.A.	(1974)	150 (330 10^3 lb)		Radding et al. (1977)
p-dimethylamino-azobenzene	(67)	Japan	(1973)	5.5		IARC (1975)
		U.S.A.	(1971)	4.5 (10 10^3 lb)		Radding et al. (1977)
		U.S.A.	(1974)	450 (10^6 lb)		NTP (1983)
o-aminoazotoluene	(70)	F.R.G.		(several hundred kg/y)		IARC (1975)
		U.S.A.	(1973)	209 (450 10^3 lb)		Radding et al. (1977)

[a] as hydrochloride
[b] estimated
[c] as sulphate
[d] 80/20 TDI mix
[e] for U.S.A. sales/import volumes of benzidine dyes (see Table 3)
[f] production exclusive of benzidine produced *in situ* for dye manufacture
[g] domestic consumption

shown in Table 3. The available information is for developed countries only and indicates that aromatic amines may be imported into countries in which they are not manufactured or were not manufactured for certain years. It has not been possible to identify all manufacturing countries and concern has been expressed that not all the countries producing some of the aromatic amines for import by non-producers may have sufficiently stringent controls to protect employee health and safety (Cartwright, 1983).

Table 3 Estimated quantities of benzidine-dyes and pigments in the U.S.A. 1975–1978

Type of dye	Quantity ($\times 10^6$ kg)			
	1975	1976	1977	1978
Benzidine-based:				
Sales	1.9	3.0	2.1	0.9
Imports	0.4	0.3	0.6	0.7
3,3′-Dichlorobenzidine-based:				
Sales	3.8	5.3	5.8	—
Imports	0.05	0.05	0.02	0.01
3,3′-Dimethylbenzidine-based:				
Sales	0.9	1.0	0.8	1.3
Imports	0.05	0.05	0.05	0.05
3,3′-Dimethoxybenzidine-based:				
Sales	0.2	0.2	0.2	0.2
Imports	0.05	0.05	0.05	0.05

Adapted from EPA (1979)

2. USES

2.1 Current and historical uses

Table 4 summarizes reported uses both past and present of several aromatic amines. Major commercial users are the dye, pharmaceutical, rubber, pesticides and plastics industries. Some countries have reportedly ceased using some of the compounds. Over the last decade, for example, several manufacturers in the U.S.A. have stopped synthesizing benzidine-based dyes and pigments and have replaced them with phthalocyanine, *o*-toluidine (2), 3,3′-dimethoxybenzidine (48), phenylenediamine and dioxyazine-type dyes (NIOSH, 1980). Similarly, although 2-naphthylamine (58) may be used as an intermediate in the production of dyes and antioxidants, its use is now generally prohibited or severely restricted. 2-Amino-1-naphthalene sulphonic acid is frequently substituted in the manufacture of dyes, and the antioxidant *N*-phenyl-2-naphthylamine (60) is now usually prepared from the condensation of aniline (1) and 2-naphthol rather than from 2-naphthylamine (IARC, 1978). Chlornaphazine (63) has been used as a chemotherapeutic agent in the treatment of leukaemia and Hodgkin's disease, and also in the control of polycythaemia vera. However, it is no longer thought to have significant therapeutic use (IARC, 1974).

Table 4 Summary of reported commercial uses (past and present) of aromatic amines

Compound	Compound No.	Dye and pigment intermediate	Other intermediates	Textile dyeing	Leather dyeing	Miscellaneous dyeing applications	Hair dyes and hair colourants	Soaps, cosmetics, perfumes and pharmaceuticals	Food processing	Rubber manufacture	Plastics (polymers) manufacture	Component in the production of fire-resistant fibres	Analytical reagent	Security printing	Pesticide/herbicide production	Anti-oxidant (other than rubber and plastics manufacture)	Other
Anilines																	
aniline[a,b]	(1)	+						+		+	+				+		+
o-toluidine[a,c]	(2)	+		+						+			+				+
m-toluidine[d]	(3)		+														
p-toluidine[d]	(4)	+															
2,4-xylidine[e]	(5)	+															+
2,5-xylidine[e]	(6)	+															
2,4,5-trimethylaniline[a,d]	(10)	+															
2,4,6-trimethylaniline[a]	(11)	+						+									
o-anisidine[a]	(12)	+						+									
p-anisidine[a]	(13)	+						+					+				
m-cresidine[a]	(14)	+															
p-cresidine[a]	(15)	+															
anthranilic acid[e]	(16)	+	+					+	+								+
p-aminobenzoic acid[e]	(17)	+	+					+									+
4-amino-2-nitrophenol[e]	(24)					+	+										
5-nitro-*o*-anisidine[a]	(25)	+															
3,4-dichloroaniline[f]	(28)														+		
2,4,6-trichloroaniline[d]	(29)	+															
p-chloro-*o*-toluidine[e]	(30)	+	+	+											+		

Table 4/contd

Compound	Compound No.	Dye and pigment intermediate	Other intermediates	Textile dyeing	Leather dyeing	Miscellaneous dyeing applications	Hair dyes and hair colourants	Soaps, cosmetics, perfumes and pharmaceuticals	Food processing	Rubber manufacture	Plastics (polymers) manufacture	Component in the production of fire-resistant fibres	Analytical reagent	Security printing	Pesticide/herbicide production	Anti-oxidant (other than rubber and plastics manufacture)	Other
Phenylenediamines																	
o-phenylenediamine[d]	(31)	+															
m-phenylenediamine[c,e]	(32)	+	+	+			+			+		+					+
p-phenylenediamine[c]	(33)	+	+	+		+	+			+	+						+
2,4-diaminotoluene[e]	(34)	+	+	+	+	+	+				+						
2,5-diaminotoluene[e]	(35)	+				+	+										
2,4-diaminoanisole[c,e]	(36)	+	+		+	+	+										+
N-phenyl-*p*-phenylenediamine[g]	(38)						+						+				
1,2-diamino-4-nitrobenzene[c,e]	(40)						+										
1,4-diamino-2-nitrobenzene[c]	(41)					+	+										
4-chloro-*o*-phenylenediamine[a]	(42)	+															+
4-chloro-*m*-phenylenediamine[a]	(43)	+								+							
2-chloro-*p*-aminoaniline[g]	(44)						+										

Table 4/contd

Compound	Compound No.	Dye and pigment intermediate	Other intermediates	Textile dyeing	Leather dyeing	Miscellaneous dyeing applications	Hair dyes and hair colourants	Soaps, cosmetics, perfumes and pharmaceuticals	Food processing	Rubber manufacture	Plastics (polymers) manufacture	Component in the production of fire-resistant fibres	Analytical reagent	Security printing	Pesticide/herbicide production	Anti-oxidant (other than rubber and plastics manufacture)	Other
Benzidines																	
benzidine[h,i,j]	(45)	+[6]								+	+		+	+			+
3,3′-dimethylbenzidine[i,j]	(46)	+											+				
2,2′-dimethylbenzidine[i,j]	(47)	+[8]															
3,3′-dimethoxybenzidine[h,i,j,k]	(48)	+[6]									+[3]		+				
3,3′-diaminobenzidine[i]	(49)											+					
3,3′-dichlorobenzidine[h,i,j]	(50)	+[7]								+							
2,2′-dichlorobenzidine[i]	(51)	+															
2,2′,5,5′-tetrachlorobenzidine[a]	(52)	+															
4,4′-Diaminodiphenylmethanes																	
4,4′-methylenedianiline[k,l,m]	(53)	+	+					+		+	+[1,2]		+			+	+
4,4′-methylenebis (2-chloroaniline)[k,n]	(54)										+						
4,4′-methylenebis (2-methylaniline)[k,l]	(55)	+									+[3]						
4,4′-methylenebis (*N*,*N*-dimethylaniline)[a,o]	(56)	+					+						+				

Table 4/contd

Compound	Compound No.	Dye and pigment intermediate	Other intermediates	Textile dyeing	Leather dyeing	Miscellaneous dyeing applications	Hair dyes and hair colourants	Soaps, cosmetics, perfumes and pharmaceuticals	Food processing	Rubber manufacture	Plastics (polymers) manufacture	Component in the production of fire-resistant fibres	Analytical reagent	Security printing	Pesticide/herbicide production	Anti-oxidant (other than rubber and plastics manufacture)	Other
Naphthylamines																	
1-naphthylamine[b,k,p]	(57)	+	+					+		+					+	+	
2-naphthylamine[b,k,p]	(58)	+[r]	+							+					+	+	
N-phenyl-2-naphthylamine[b,e]	(60)	+	+							+	+					+	+
1,5-naphthalenediamine[a,p]	(61)	+	+								+						
1,8-naphthalenediamine[p]	(62)					+[s]											
chlornaphazine[k]	(63)							+									
Aminoazobenzenes																	
p-aminoazobenzene[q]	(65)	+				+											
2,4-diaminoazobenzene[c]	(66)			+	+	+											
p-dimethylaminoazobenzene[q]	(67)			+		+							+				
o-aminoazotoluene[q]	(70)					+											
diacetylaminoazotoluene[q,r]	(71)							+									

Table 4/contd

Compound	Compound No.	Dye and pigment intermediate	Other intermediates	Textile dyeing	Leather dyeing	Miscellaneous dyeing applications	Hair dyes and hair colourants	Soaps, cosmetics, perfumes and pharmaceuticals	Food processing	Rubber manufacture	Plastics (polymers) manufacture	Component in the production of fire-resistant fibres	Analytical reagent	Security printing	Pesticide/herbicide production	Anti-oxidant (other than rubber and plastics manufacture)	Other
Miscellaneous																	
4-aminobiphenyl[c]	(72)									+							

[a] IARC (1982a)
[b] Fishbein (1980)
[c] Fishbein (1979)
[d] Weisburger et al. (1978)
[e] IARC (1978)
[f] Sundström (1980)
[g] Van Duuren (1980)
[h] IARC (1982b)
[i] Ferber (1978)
[j] EPA (1979)
[k] IARC (1974)
[l] Fishbein (1977)
[m] Moore (1978)
[n] NTP (1983)
[o] Marzulli et al. (1978)
[p] Dressler (1978)
[q] IARC (1975)
[r] Merck (1968)

[1] As both parent compound and diisocyanate derivative
[2] As methylenebiscyclohexylamine and diisocyanate derivative
[3] As the diisocyanate derivative
[4] Other reagents now substituted
[5] As phthaloperinone
[6] Mainly 'Direct' azo dyes
[7] In the form of its dihydrochloride
[8] Limited to cellulosic dyeing

The major use of benzidine (45) and its congeners is in the production of dyes and pigments for various purposes. Some of these dyes have been implicated as possible human carcinogens and therefore warrant further discussion.

Benzidine-based dyes have been used mainly in the leather, textile and paper industries, as well as by beauticians, craft workers and the general public. More than 30 benzidine-based dyes have been reported to have commercial importance in the U.S.A., 13 of which were imported (NIOSH, 1980). Some of the more widely used dyes are shown in Table 5.

3,3′-Dichlorobenzidine (50) is almost exclusively used in the manufacture of red, orange and yellow pigments. 3,3′-Dichlorobenzidine pigments are reportedly used in the colouring of plastic resins, lacquers, printing inks, paints and toy enamels and in textile and wallpaper printing (EPA, 1979). Dyes and pigments prepared from two other chlorobenzidine compounds, 2,2′-dichlorobenzidine (51) and 2,2′,5,5′-tetrachlorobenzidine (52), have been reported in the literature to be of little commercial importance (Ferber, 1978; IARC, 1982a).

3,3′-Dimethylbenzidine (46) is used to produce a number of dyes which have been used for colouring products similar to those listed above for benzidine- and 3,3′-dichlorobenzidine-based dyes (EPA, 1979). 3,3′-Dimethylbenzidine has been reported to be used in 24 dyes in the U.S.A., the most important of which are Direct Reds 2 and 39 and Direct Blue 25 (Ferber, 1978; EPA, 1979). 2,2′-Dimethylbenzidine (47) has been used in the production of a few dyes, none of which is of major importance (Ferber, 1978).

3,3′-Dimethoxybenzidine (48) has been reported to be used in 36 dyes in the U.S.A. used for dyeing leather, paper, plastics, rubber and textiles (EPA, 1979). Following demethylation of the methoxy groups in alkali, the resulting dihydroxy compound can be chelated with copper which imparts the dye with light-fast properties (Ferber, 1978).

2.2 Regulations and exposure limits

Many of the aromatic amines discussed herein are subject to regulations and restrictions which vary among different countries, examples of which are summarized in Table 6. Detailed descriptions of regulations are summarized for a few aromatic amines in the IRPTC Legal File (1983). Montesano & Tomatis (1977) also have reviewed some of the legislation in several countries concerning carcinogenic amines. Table 6 is intended only to give an indication of the differences in regulations in some countries and is neither exhaustive nor representative of the most recent situation. Several countries specify some of the aromatic amines as carcinogens (see Table 7) with no assigned occupational exposure limits. In these circumstances, standards may be set to limit or minimize occupational exposure and in some cases employee exposure may not be permitted, i.e. exposure limits must be effectively zero. In the United Kingdom, medical surveillance and registration of employees exposed to certain chemicals is required. In addition, the EEC restricts the use of some aromatic amines in cosmetics including aniline (1) and *o*-phenylenediamine (31), *m*-phenylenediamine (32) and *p*-phenylenediamine (33) and prohibits the use of benzidine. Czechoslovakia prohibits the use of *m*- and *p*-phenylenediamine in cosmetics (IRPTC, 1983). An IARC Manual (Egan et al., 1981) gives selected methods for the monitoring and analysis of aromatic amines.

Table 5 Uses of benzidine-based dyes and pigments commercially available on the U.S.A. market in 1979

		Dyeing									Printing						Miscellaneous					
Name	Compound No.	Cotton	Wool	Silk	Nylon	Viscose	Leather	Plastics	Paper	Soap	Cellulosics	Nylon	Silk	Viqoureux	Aqueous inks	Biological stain	pH indicator	Typewriter ribbons	Wood stain	Hair dye formulations	Heavy metal salts used as pigments	
Acid Red 85*		+	+	+	+	+	+							+								
Direct Black 4*		+	+	+	+		+		+													
Direct Black 38	(76)	+	+	+			+	+							+	+		+	+			
Direct Blue 2		+					+		+													
Direct Blue 6	(77)	+		+			+		+						+	+						
Direct Brown 1		+	+	+	+		+		+											+		
Direct Brown 2*		+	+	+	+		+		+												+	
Direct Brown 6		+	+	+			+		+													
Direct Brown 31							+		+		+									+	+	
Direct Brown 59		+	+	+			+															
Direct Brown 74		+	+	+			+															
Direct Brown 95	(78)	+	+	+			+	+	+												+	
Direct Brown 154		+	+	+			+		+		+		+									
Direct Green 1		+	+	+	+		+		+		+	+	+		+							
Direct Green 6		+	+	+	+				+	+		+			+						+	
Direct Green 8		+	+	+	+		+		+													
Direct Orange 8		+	+	+	+				+													
Direct Red 1*		+	+	+	+		+		+												+	
Direct Red 28**		+	+	+					+		+					+	+					
Direct Red 37		+	+	+	+				+		+	+										
Direct Violet 1		+	+	+			+		+							+						
Direct Violet 22		+	+	+	+		+															

From EPA (1979), NIOSH (1980), Van Duuren (1980), IARC (1982b)

*Also reported as a colourant for printing textiles (material(s) not specified).

** Also known as Congo Red, this has been listed as a food colour (Combes & Haveland-Smith, 1982)

Note: This table does not include any dyes or pigments containing benzidine congeners (i.e. substituted benzidines)

Table 6 Regulations on the manufacture and use of some carcinogenic aromatic amines

Compound	Compound No	Prohibited			Closed/isolated systems required		Subject to authorization
		I	M	U	M	U	
Anilines							
o-toluidine	(2)						
Belgium					+		+
Japan					+		+
Benzidines							
benzidine	(45)						
Belgium					+[1]		+[1]
F.R.G							−[2]
Japan		+[1,3]	+[1,3]	+[1,3]			
Sweden			+[1]	+[1]			
U.K. and Eire		+[1,4]	+[1,4]	+[1,4]			+[5]
U.S.A.					+[6]	+[6]	
3,3′-dimethylbenzidine	(46)						
Sweden							+
3,3′-dimethoxybenzidine	(48)						
F.R.G.					+		+
Sweden							+
3,3′-dichlorobenzidine	(50)						
Belgium					+		+
F.R.G.							+[7]
Japan					+		+
Sweden							+
U.S.A.					+[1,2]	+[1,2]	
U.S.S.R.			+				
4,4′-Diaminodiphenylmethanes							
4,4′-methylenebis-(2-chloroaniline)	(54)						
Sweden							+
Naphthylamines							
1-naphthylamine	(57)						
Belgium					+		+
Japan					+		+
Sweden							+
2-naphthylamine	(58)						
Belgium					+		+
Japan		+[3]	+[3]	+[3]			
Sweden			+	+			
U.K. and Eire		+[4]	+[4]	+[4]			+[5]
U.S.S.R.			+				

Table 6/contd

Compound	Compound No	Prohibited			Closed/isolated systems required		Subject to authorization
		I	M	U	M	U	
Aminoazobenzenes							
p-dimethylaminoazo-benzene	(65)						
Belgium					+		+
Sweden			+	+			
U.S.S.R			+				
o-aminoazotoluene	(70)						
U.S.S.R.			+				
Miscellaneous							
4-aminobiphenyl	(72)						
Belgium					+		+
Japan		+[3]	+[3]	+[3]			
Sweden			+	+			+[5]
U.K. and Eire		+[4]	+[4]	+[4]			
2-acetylaminofluorene	(73)						
Belgium					+		+
Sweden			+	+			
auramine	(74)						
Japan					+		
Sweden							+
magenta	(75)						
Japan					+		

From Montesano & Tomatis (1977), IRPTC (1983), Swedish National Labour Protection Board (1984)
I import
M manufacture
U use
[1] also applies to salts
[2] applies to use and handling of >0.1%
[3] products with <1% exempt
[4] substances with <1% as by-product exempt
[5] special exemption may be granted for medical or scientific research
[6] solid or liquid mixtures <0.1% exempt
[7] applies to use and handling of >1%

3. ENVIRONMENTAL SOURCES AND FATE

3.1 Sources

With the exception of anthranilic acid (16) and *p*-aminobenzoic acid (17), the aromatic amines discussed in this document do not occur naturally in the environment. They may, however, be present in waste streams or waste gases from plants in which they are produced or used. Primary aromatic amines have been identified in synthetic fuel oils and coal gasification by-products (Haugen et al., 1982). Mutagenic materials including anilines were found to remain in the ground water from an underground coal gasification zone for at least two years (Timourian et al., 1982).

Monocyclic aromatic amines identified in effluents from chemical plants include aniline (1), *o*-toluidine (2), *o*-anisidine (12) and *p*-anisidine (13) and chloroanilines. The latter have been found in sewage sludge in four out of nine cities in the U.S.A. (Parris, 1980; IARC, 1982a). Aniline and *o*- and *p*-anisidine have also been identified in effluents from oil refineries and aniline has additionally been found in effluents from oil shale recovery and coal conversion plants. Anilines and substituted anilines may also be released into the environment by the combustion of plastics or urethane products or as the reduced form of nitrobenzene products (Fishbein, 1980). *o*-Toluidine has been detected in tar produced by the low temperature carbonization of coal and in the gasoline fraction of hydrocracked Arlan petroleum (IARC, 1982a). Anthranilic acid is both a metabolite of and, in some micro-organisms, an intermediate in the synthesis of tryptophan. *p*-Aminobenzoic acid is also a metabolite of several micro-organisms and is found naturally in some yeasts. Although *p*-chloro-*o*-toluidine (30) does not occur naturally, it may be formed from the enzymatic decomposition of the pesticide chlordimeform in the leaves of some plants (IARC, 1978).

Anilines (primarily halogenated) are known to be produced in soil from the degradation of some herbicides including phenylcarbamate, phenylurea and acylanilide herbicides. Further transformation may lead to the formation of azobenzenes and other polymers (Bartha et al., 1968).

Manufacturing and processing plants for benzidine-based dyes and pigments are reported to be the major source of release of benzidine (45) and its congeners (EPA, 1979). Airborne release can occur during weighing, loading and mixing operations where particles may be discharged into the air outside the plant (EPA, 1979). In the U.S.A. significant accidental releases have occurred (Fishbein, 1979), although the majority of discharges of benzidine in waste water from production facilities were less than 0.5 kg per day. Benzidine and congeners may also be released into waste streams both as a residual dye contaminant and/or by subsequent *in situ* bacterial and chemical reduction of the respective parent dyes (IARC, 1982b). It was estimated (from production figures) that about 9,000 kg of benzidine was released from dye production in the U.S.A. during 1972. The possibility of dyes being released from landfill sites has also been described (EPA, 1979).

Contamination of soil sediments and waste water in the vicinity of a 4,4′-methylenebis(2-chloroaniline) (54) manufacturing plant may have been due to dust from the manufacturing process or transport of the material away from the plant (Voorman, 1981). Since 4,4′-methylenebis(2-chloroaniline) sublimes rapidly at 200°C and was melted during its use at the plant, sublimation may also have contributed to environmental contamination (Zabik, 1982).

Both 1- (57) and 2-naphthylamine (58) have been identified in a mixture derived

from a process stream of a coal gasification pilot plant (Haugen et al., 1982) and have also been reported in effluent from certain dyestuff factories (Fishbein, 1980). Naphthylamine isomers may be formed from pyrolysis of nitrogen containing organic matter and both have been found in coal tar (IARC, 1974).

3.2 Fate

Radding et al. (1977), Fishbein (1980) and Parris (1980) have reviewed the possible environmental fate of aromatic amines.

3.2.1 Soil and organic matter

Several studies have demonstrated the absorption of aromatic amines by soils. Soil transformation products may be both more readily absorbed (Pillai et al., 1982) and more stable (Medvedev & Davidov, 1981) than the parent amine. The amount of organic matter present has been shown to make a significant contribution to the absorption of anilines in soil (Moreale & van Bladel, 1976; Pillai et al., 1982).

Anilines and derivatives may condense to form azobenzenes and other polymeric species. In practice, the high levels of aromatic amines used in laboratory experiments probably influence the amount of condensation products isolated (Parris, 1980) and from the levels of anilines liberated by field application of related herbicides only small quantities of polymeric material would be formed (Hsu & Bartha, 1974a). The ultimate degradation products of aniline (1) are CO_2 and NH_3 (Pillai et al., 1982).

In a study on the transformation in soil of chemicals in industrial sewage from the coke industry (a potential nitrogen fertilizer), aniline applied at 500 ppm (500 mg/kg) was found to be relatively slowly destroyed in soil (not detectable after 13 d at 19°C) and to form unstable transformation products. In contrast, *o*- (2) and *p*-toluidine (4) and 2,4- (5) and 2,6-xylidine (7) were rapidly transformed (not detectable after 3–9 d) to long-lived products (detectable >90 d) under the same conditions (Medvedev & Davidov, 1981).

Herbicide-derived chloroanilines are rapidly bound to soil. Two weeks after soil application of [^{14}C]-*p*-chloroaniline (27) at 500 ppm, only 41% of the total applied radioactivity was extractable (Bordeleau & Bartha, 1972). Experiments with radiolabelled *p*-chloroaniline, which may be extracted from soil more efficiently with chloroaniline solutions than with pure water, imply that the amine is competitively bound to specific sites in the soil (Parris, 1980). Hsu & Bartha (1974a) have proposed that most of the chloroaniline moiety derived from herbicide decomposition is bound to organic matter in soil, both by physical adsorption and reversible (possibly via imines) and irreversible chemical binding. Based on the rate of release of $^{14}CO_2$ from soil treated with ring-labelled 3′,4′-dichloropropionanilide (propanil), a residence time of 2–4 years for soil-bound dichloroanilines in soil was estimated (Hsu & Bartha, 1974a) and soil residence times of up to 10 years have been predicted (Hsu & Bartha, 1974b). Several authors have expressed concern about the biological activity of humus-bound aromatic amine residues which are non-extractable by normal monitoring methods. As well as binding to humic acids, incorporation into plant lignin has been demonstrated with substituted anilines (Parris, 1980) and aniline and its analogues may form charge-transfer complexes with clays (Fishbein, 1980).

3,3′-Dichlorobenzidine (50) adsorbs readily to a variety of aquatic sediments and

becomes more tightly bound with time (EPA, 1979). Benzidine in soil is thought to be adsorbed to humic material and clays, and rapidly immobilized (Fishbein, 1980). The Fe^{3+}, Al^{3+} and Cu^{2+} ions which are readily available in some clay soils are believed to oxidize benzidine (45) very rapidly (EPA, 1979). The adsorption of several benzidine-based dyes to activated sewage sludge ranged from 50-92%. Two 3,3′-dimethoxybenzidine-based dyes (Direct 1 and 15) showed a 92% and 90% adsorption respectively.

4,4′-Methylenebis(2-chloroaniline) (54) is also bound rapidly to soil. Approximately 50% of ^{14}C ring-labelled compound applied over a range of concentrations was absorbed by soil samples within 24 h. By 24 w <20% of the applied radioactivity was extractable, <6% as the parent amine. The absorption isotherm also indicated a strong affinity for soil (Voorman, 1981).

In the study by Medvedev & Davidov (1981), 2-naphthylamine (58) applied to soil at 500 ppm (500 mg/kg) was stable (detectable >90 d) and was probably not susceptible to transformation.

3.2.2 Water and air

Transport to the atmosphere from water or sorption to sediments or organisms from water would be expected to be slight for most simple aromatic amines as they have relatively low vapour pressures and high solubility. The high lipophilicity of some azobenzenes, however, may lead to absorption by sediments having a significant organic content (Radding et al., 1977). Aromatic amines and aminoazo dyes would be expected to be relatively rapidly oxidized in air (half-life ~ 100h) or water (half-life ~ 8 h) (Radding et al., 1977). An estimated half-life of aniline in river water of 2–3 days has been reported (Fishbein, 1980) and the half-life of benzidine (45) in air has been approximated to be in the region of 1 day: its fate has been predicted to involve photolysis and oxidation by ozone (EPA, 1979). Chlorine substitution should have little or no effect on the rates of oxidation in the atmosphere but may retard oxidation in water (Radding et al., 1977). Chlorination of water contaminated with aromatic amines may lead to the formation of products which have increased toxicity and may adversely affect the water's organoleptic properties (Shtannikov & Lutsevich, 1982).

3.2.3 Biodegradation

Biodegradation is likely for many aromatic amines and azobenzenes. Benzidine derivatives and dyes, however, have been shown to possess some bacteriostatic properties (Radding et al., 1977). Formation of azobenzenes and other polymeric products from anilines applied to soils has been proposed to be mediated by microbial oxidases and peroxidases (Bartha et al., 1968; Bordeleau & Bartha, 1972). However, similar derivatives have also been demonstrated from aniline in sterile soils (Pillai et al., 1982). Nitrite ions, generated from bacteria containing nitrate reductase enzyme systems, may also react to produce condensation products via diazonium ion formation (Pillai et al., 1982). Aoki et al. (1983) isolated 20 strains of aniline-assimilating bacteria in soil when aniline (1) was provided as the sole source of carbon and nitrogen. Acetylation of some aromatic amines by soil micro-organisms has been reported but it has been suggested that this may be an artefact of the extraction and analytical procedures used (Pillai et al., 1982).

Benzidine (45) has been reported to be resistant to bio-oxidation by unacclimated micro-organisms in activated sludge. Other studies suggest that although benzidine is not biodegradable at concentrations of 1 mg/litre, it is at 0.05 mg/litre. It has been demonstrated that acclimation of sludge micro-organisms can be achieved readily with benzidine concentrations up to 5 mg/litre, and that complete oxidation of benzidine at levels of 1 mg/litre can be achieved by acclimated sludges with less complete oxidation at higher concentrations (EPA, 1979; Fishbein, 1980). Benzidine (50 mg/litre) was not degraded by soil bacteria when incubated with river water for up to 9 weeks (at 37°C) (Yoshida et al., 1981).

In one study 4,4'-diamino-3,3'-dichlorobenzophenone was found as a product of aerobic microbial metabolism of 4,4'-methylenebis(2-chloroaniline) (54) within 3 days of its application to soil. Limited microbial oxidation of ^{14}C-ring-labelled compound to $^{14}CO_2$ also occurred, implying some ring cleavage (Voorman, 1981).

In a laboratory sewage sludge system *N*-phenyl-2-naphthylamine (60) was biotransformed into 2-naphthylamine (58) with decreasing efficiency for increasing *N*-phenyl-2-naphthylamine levels (2–8 mg/litre (ppm)). The transformation appeared to be dependent solely on microbial activity since no 2-naphthylamine was formed in sterilized sludge with 2 mg/litre (2 ppm) *N*-phenyl-2-naphthylamine added. Further biodegradation of 2-naphthylamine was apparently limited (Fishbein, 1980).

3.2.4 Photolysis

3,3'-Dichlorobenzidine (50) is rapidly degraded in aqueous solution by the action of natural or simulated sunlight (EPA, 1979). Benzidine (45) and 3-chlorobenzidine are intermediates in this process. Photodegradation was slower in organic solvents. Benzidine absorbs strongly in the solar region (below 350 nm) but no photochemistry has been reported (Radding et al., 1977). 4,4'-Methylenebis(2-chloroaniline) (54) is degraded by U.V. radiation. Photo-oxidation has been reported only in the top several mm of soil (Zabik, 1982). Azobenzenes have a strong chromophore and absorb visible and near U.V. solar radiation, therefore photochemical transformations would be expected. However, both photo-reduction and photo-oxidation apparently only occur slowly (Radding et al., 1977).

3.2.5 Bioconcentration and bioaccumulation

Aromatic amines can and have entered the foodchain (Parris, 1980). Although Probst et al. (1967) found that most crops absorbed almost none of the herbicide trifluralin (1,1,1-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine), Still et al. (1980) found that 3,4-dichloroaniline (28), a degradation product of the herbicide propanil, was absorbed by rice plants and 0.4 ppm could be found in the grains.

Both benzidine (45) and 3,3'-dichlorobenzidine (50) have been found to be rapidly bioconcentrated in blue gill sunfish. Based on total ^{14}C-residues, a bioconcentration factor of about 500 was observed for [^{14}C]-3,3'-dichlorobenzidine. Elimination of radioactivity was incomplete upon transfer of the fish to water free from 3,3'-dichlorobenzidine (Fishbein, 1981). Benzidine neither bioaccumulated nor was transferred through food chains to higher levels when tested in laboratory model ecosystems (Fishbein, 1979).

Voorman (1981) demonstrated that although 4,4'-methylenebis(2-chloroaniline)

(54) could be absorbed by plant leaves, it did not migrate from the point of application. The amount absorbed by the leaf may be related to the thickness of the surface wax. Although ^{14}C label associated with the roots of plants, grown in either aqueous culture medium or soil contaminated with radiolabelled 4,4'-methylenebis (2-chloroaniline), could not be removed by rinsing, there was little translocation of radioactivity to the shoots.

4. ENVIRONMENTAL AND OCCUPATIONAL LEVELS AND EXPOSURES

4.1 General population

4.1.1 Anilines

Monocyclic aromatic amine contamination of river waters has been reported in several countries including the F.R.G., where contaminants found include aniline (1) (0.5–3.7 μg/litre) attributed to industrial chemical wastes, *N*-methylaniline (8) (0.8–1.1 μg/litre) and *o*-toluidine (2) (0.3-1 μg/litre) (Neurath et al., 1977); The Netherlands, where annual average concentrations in several rivers during 1971 were determined for aniline (<0.1–4 μg/litre) and some chlorinated anilines (<0.1–1 μg/litre) (Greve & Wegman, 1975); and the U.S.A., where aniline has also been identified in finished drinking water. *o*-Toluidine has been found in sea water (IARC, 1982a).

Levels of monocyclic aromatic amines reported in a survey of foods for human consumption included aniline (<0.1–30.9 mg/kg), *N*-methylaniline (0.2–37.9 mg/kg) and toluidine (<0.1–7.2 mg/kg). Aniline was also detected in rapeseed cake (an animal feed) at 120 mg/kg (Neurath et al., 1977). Small amounts of *p*-aminobenzoic acid (17) are present in cereals, eggs, milk and meat, and it also occurs naturally in baker's and brewer's yeasts (5–6 mg/kg and 10–100 mg/kg respectively) (IARC, 1978). *p*-Aminobenzoic acid, although traditionally considered to be a member of the vitamin B complex, is not a true vitamin for any mammalian species but is a growth factor and precursor of folic acid in some bacteria (Danford & Munro, 1980). Aniline and *o*-toluidine have been detected in the volatile components of black tea. They have also both been found in tobacco smoke (50.6–577 ng/cigarette and 23.3–213 ng/cigarette respectively), as has *o*-anisidine (12), and have been identified among steam volatiles from the distillation of a type of tobacco leaf, as have 2,5-xylidine (6) and 2,4,6-trimethylaniline (11) (IARC, 1978, 1982a). NIOSH has estimated that in the U.S.A. more than 15 million people may be exposed to phenylenediamines used in hair and fabric dyes or photographic development fluids (Milman & Peterson, 1984).

4.1.2 Benzidines

The general public is exposed mainly to finished benzidine dyes and pigments after they have been applied to textiles, leather and other products. Although these dyes and pigments in finished products are considered to be "fast" (i.e. they do not leach out), Yoshida et al. (1971) demonstrated bacterial liberation of benzidine (45) from Direct Black 38 (76) (a benzidine-based dye) after the dye had been attached to cotton

cloth. *E. coli* (a common bacteria found on the skin) has not only been shown to liberate benzidine from Direct Black 38 but has also been shown to be unusually resistant to the bacteriostatic effect of dyes in general. Direct exposure to both benzidine or congeners, present as residual unreacted starting material, is also possible (EPA, 1979).

In a report by the EPA (1979), 15 consumer retail dyes purchased in arts and crafts shops were analysed for benzidine-, 3,3′-dimethylbenzidine- and 3,3′-dimethoxybenzidine-based dyes. Of these dyes, nine appeared to be wholly or predominantly benzidine-based and five 3,3′-dimethylbenzidine-based. Only one dye contained no detectable benzidine or benzidine congener.

Effluents from leather and textile factories making 'heavy' use of benzidine-based dyes contained 0.25 (estimated) and 3.5 (average) μg/litre residual benzidine respectively (EPA, 1979).

Benzidine concentrations of up to 233 μg/litre were measured downstream from a dye plant in Japan. This was attributed to chemical reduction of the dye and pigment molecules to benzidine when hydrogen sulphide or sulphur dioxide was present in the water. Benzidine and 3,3′-dichlorobenzidine (50) have been detected in river and surface waters and raw sewage effluents (IARC, 1982b).

Analyses of purge wells and seepage water near a lagoon receiving wastes from a 3,3′-dichlorobenzidine manufacturing plant were found to contain 0.13–0.27 mg/litre 3,3′-dichlorobenzidine. It would appear that there is no information available regarding the possible dissipation or levels of 3,3′-dimethylbenzidine (46) and 3,3′-dimethoxybenzidine (48) in the environment (IARC, 1982b).

The possibility of exposure through the consumption of contaminated fish has also been reported. ^{14}C residues from ^{14}C-labelled 3,3′-dichlorobenzidine were found to be rapidly accumulated by fish (see Section 3.2.5.) in both the edible and non-edible portions (Fishbein, 1981).

4.1.3 4,4′-Diaminodiphenylmethanes

Acute intoxication of at least 84 members of the public by 4,4′-methylenedianiline (53) occurred following the ingestion of wholemeal bread baked with accidentally contaminated flour. In a sample of flour a 4,4′-methylenedianiline level of 13 mg/kg was detected. A sample of bread was estimated to contain 2,600 mg/kg amine (0.26%) when the moisture content of the bread was 11.5%. The bread had a considerably higher level since it was baked from flour at the top of a storage bin where the contamination was greatest whereas the flour sample was taken from the bottom of the bin (Kopelman et al., 1966a).

Contamination of the general population also occurred in the vicinity of a 4,4′-methylenebis(2-chloroaniline) (54) production plant. The area surrounding the plant was contaminated with the chemical. The amine was found in all soil samples within a three- to four-mile radius of the plant. Levels on public roads were in excess of 500 mg/kg, deposits in eaves troughs were as high as 400 mg/kg sludge from the waste water treatment plant contained up to 86 mg/kg of the amine and sludge from the industrial lagoon 2,000 mg/kg. Levels of up to 18 mg/kg were found in domestic vacuum cleaners and the amine was also found in the urine of children resident in the contaminated area and in the families of employees at the plant. One suggested mode of domestic contamination was from the transfer of 4,4′-methylenebis(2-chloroaniline) to the clothing of family members when

contaminated work clothes were washed with domestic laundry (NTP, 1983; Manis et al., 1984). It has been suggested that residual levels of 4,4′-methylenebis(2-chloroaniline) may be present in consumer products but no data are available describing levels of such impurities or the potential for consumer exposure (NTP, 1983).

4.1.4 Naphthylamines

The general population may be exposed to 1- (57) and 2-naphthylamines (58) in cigarette smoke. A simulated smoking experiment demonstrated mainstream levels of 1-naphthylamine of 0.03μg and 2-napthylamine of 0.02μg per cigarette (Hoffmann et al., 1969). It has been estimated that a heavy smoker (⩾40/d) might inhale up to 1 μg of 2-naphthylamine per day (Nutt, 1983).

When butadiene rubber was heated to 220°C, the evolved fumes contained 12mg/kg of N-phenyl-2-naphthylamine (IARC, 1978). Incineration of certain waste-rubber products may therefore be a potential source of exposure to naphthylamine additives.

Patients treated for polycythaemia or Hodgkin's disease with chlornaphazine (63) have been given cumulative doses of up to several hundred grams of the drug (IARC, 1974).

4.1.5 Aminoazobenzenes

The general population is exposed to azo dyes because of their ubiquitous use as synthetic colours, notably in food, pharmaceuticals and cosmetics (see Walker, 1970; Radomski, 1974; Combes & Haveland-Smith, 1982; Chung, 1983). Despite the fact that fewer azo dyes have been used in foods in recent years, the amount of such dyes consumed per person per year, in the U.S.A. for example, has been increasing over the last 20 years (Chung, 1983). Clothes fabrics coloured with Disperse dyes are also an important source of exposure to aminoazobenzenes (Sim-Davies, 1972).

4.2 Working population

Permitted occupational exposure limits for several aromatic amines in various countries are summarized in Table 7.

4.2.1 Anilines

There are few published reports on occupational exposure to monocyclic aromatic amines. Estimates of the numbers of workers potentially exposed in the U.S.A., as reported by the NTP (1983), include 13,900 to *o*-toluidine (2), 1,600 (a 1974 National Occupational Hazard Survey (NOHS) estimate) to *N,N*-dimethylaniline (9), and 1,800 (1974 NOHS estimate) to anisidine derivatives. In addition, an estimated 400,000 people, mostly hairdressers and cosmetologists, were considered to be potentially exposed to 2,4-diaminoanisole (24) in the U.S.A. (Fishbein, 1979) and NIOSH has estimated that more than 64,000 people may be potentially occupationally exposed to a group of seven phenylenediamines (Milman & Peterson, 1984). Levels of *o*-toluidine at a manufacturing plant in the U.S.S.R. were reported to be 0.5–28.6

Table 7 Occupational exposure limits

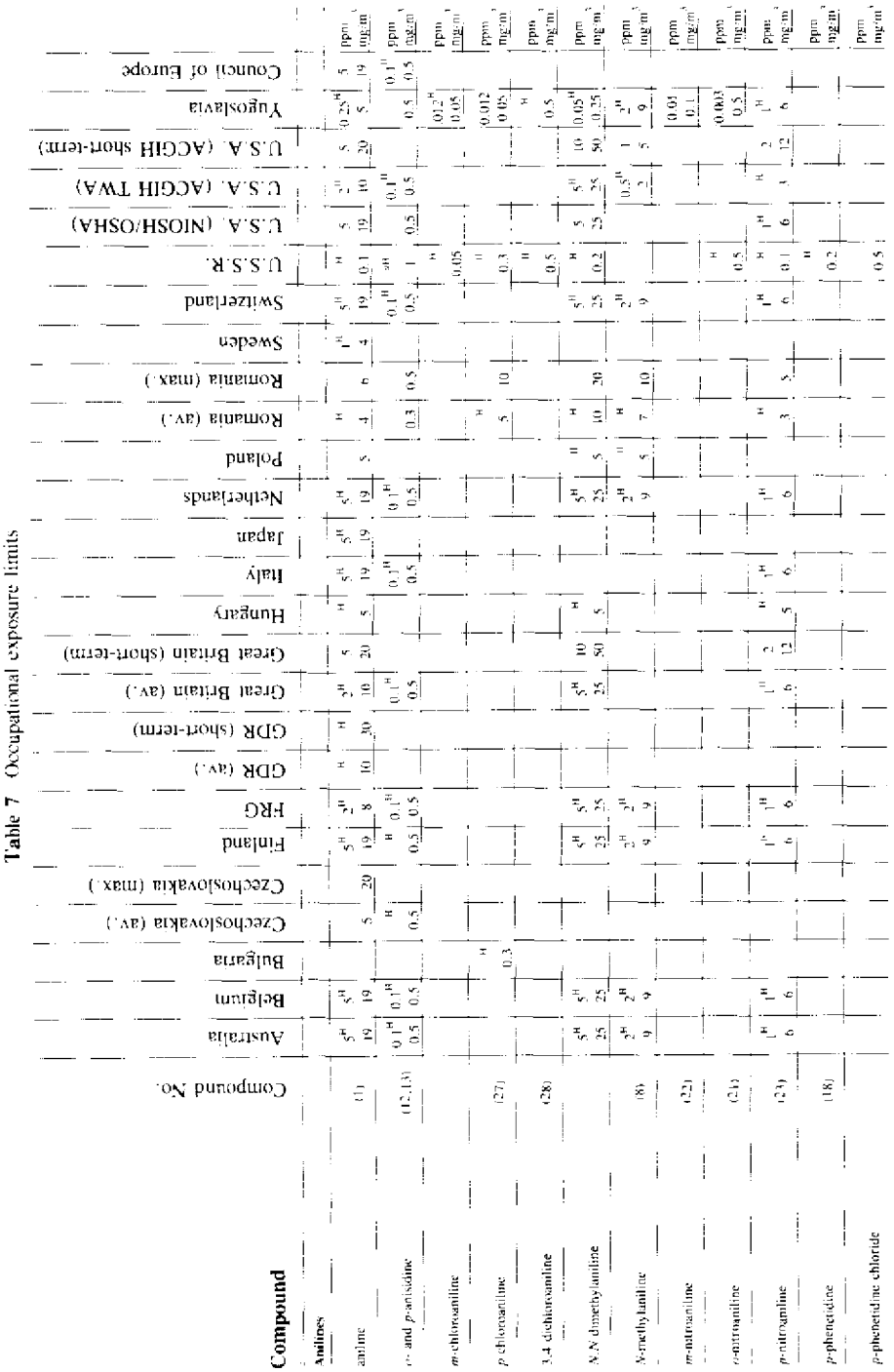

Table 7/contd

Compound No.	Australia	Belgium	Bulgaria	Czechoslovakia (av.)	Czechoslovakia (max.)	Finland	FRG	GDR (av.)	GDR (short-term)	Great Britain (av.)	Great Britain (short-term)	Hungary	Italy	Japan	Netherlands	Poland	Romania (av.)	Romania (max.)	Sweden	Switzerland	U.S.S.R.	U.S.A. (NIOSH/OSHA)	U.S.A. (ACGIH TWA)	U.S.A. (ACGIH short-term)	Yugoslavia	Council of Europe
tetranitroaniline																0.1 mg/m³										
o-toluidine (2)	5 ppm 22 mg/m³	5[C] ppm 22 mg/m³	3 ppm	5 ppm	20 ppm	5[H] ppm 25 mg/m³	5[C,R] ppm 22 mg/m³	H 10 ppm 44 mg/m³	H 20 ppm	5[H] ppm 25 mg/m³	10 ppm 50 mg/m³		2.5[C] 10 ppm		5 ppm 22 mg/m³	H 3 ppm	H 3 ppm	5 ppm		5[H] ppm 22 mg/m³	H 3 ppm	5[H] ppm 22 mg/m³	2[H] ppm 9 mg/m³		5[H] ppm 22 mg/m³	
p-toluidine (4)																					H 3 ppm					
xylidines (5,6,7)	5[H] ppm 25 mg/m³	5[H] ppm	3 ppm	5 ppm	20 ppm	5[H] ppm 25 mg/m³	4[C,R] ppm 25 mg/m³	H 10 ppm	H 20 ppm	5[H] ppm 25 mg/m³	10 ppm 50 mg/m³		2[H] ppm 10 mg/m³		5[H] ppm 25 mg/m³	H 5 ppm	H 1 ppm	2 ppm			H 3 ppm	5[H] ppm 25 mg/m³	2[H] ppm 10 mg/m³		0.6 ppm 3 mg/m³	
Phenylenediamines																										
p-phenylenediamine (33)	H 0.1 ppm 0.4 mg/m³	H 0.1 ppm	0.4 ppm			H 0.1 ppm	H 0.1 ppm		H 0.1 ppm						H 0.1 ppm	H 0.1 ppm	H 0.07 ppm 0.4 mg/m³	H 0.1 ppm	H 0.1 ppm	H 0.1 ppm		H 0.1 ppm			H 0.1 ppm	
toluenediamine (34,35)			[C]					2 ppm	4 ppm								5 ppm	10 ppm			H 2 ppm					
Benzidines and related compounds																										
2-aminobenzidine		[C]																								
4-aminobiphenyl (72)	[C] H	[C] H													[C] H					[C]			[C] H			
benzidine and salts (45)	[C] H	[C]					[C]		[C]						[C] H		[C] H			[C] H			[C] H			
benzidine production (45)		[C] H													[C] H											

Table 7/contd

Compound No.	Australia	Belgium	Bulgaria	Czechoslovakia (av.)	Czechoslovakia (max.)	Finland	FRG	GDR (av.)	GDR (short-term)	Great Britain (av.)	Great Britain (short-term)	Hungary	Italy	Japan	Netherlands	Poland	Romania (av.)	Romania (max.)	Sweden	Switzerland	U.S.S.R.	U.S.A. (NIOSH/OSHA)	U.S.A. (ACGIH TWA)	U.S.A. (ACGIH short-term)	Yugoslavia	Council of Europe	
3,3'-dichlorobenzidine (50)	C, H	C, H				C	C, H						C		C, H				CL	C, H			C				ppm mg/m³
3,3'-dimethylbenzidene (47)		C					C												CL	C			C				ppm mg/m³
4,4'-Aminodiphenylmethanes and related compounds																											
auramine (74)													C						C								ppm mg/m³
4,4'-methylenebis (2-chloroaniline) (55)	C 0.02	0.02[H]					C			0.02[H]			C 0.02		0.02				CL	0.02[H] 0.22			0.02[H] 0.22				ppm mg/m³
methylenebis (4-cyclohexylisocyanate)	0.01[M] 0.11	0.1[M] 0.11								0.01[M] 0.11			0.02[M] 0.2		0.1[M] 0.11				0.15[M] 0.11	0.01[M] 0.11							ppm mg/m³
methylenebis (phenyl-isocyanate)	0.02[M] 0.2	0.02[M] 0.2				0.02[M] 0.2	0.02[S] 0.2	0.15	0.15	0.02[M] 0.2			0.02[M] 0.2		0.02[M] 0.2				0.01[S][M] 0.1	0.02[S][M] 0.2		0.02[M] 0.2	0.02 0.2		0.02 0.2	0.02 0.2	ppm mg/m³
4,4'-methylenedianiline (53)								0.8	4													0.1[H] 0.8	0.5 4				ppm mg/m³
Naphthylamines and related compounds																											
1-naphthylamine (57)		C				C													CL				C H	C			ppm mg/m³
2-naphthylamine (58)	C	C				C	C			C			C		C			C	C	C H			C	C H			ppm mg/m³

Table 7/contd

Compound No.	Australia	Belgium	Bulgaria	Czechoslovakia (av.)	Czechoslovakia (max.)	Finland	FRG	GDR (av.)	GDR (short-term)	Great Britain (av.)	Great Britain (short-term)	Hungary	Italy	Japan	Netherlands	Poland	Romania (av.)	Romania (max.)	Sweden	Switzerland	U.S.S.R.	U.S.A. (NIOSH/OSHA)	U.S.A. (ACGIH TWA)	U.S.A. (ACGIH short-term)	Yugoslavia	Council of Europe
																									ppm mg/m³
1,5-naphthalene diisocyanate							0.02[a] 0.18																		ppm mg/m³
N-phenyl-2 naphthylamine	(60)									C															
Dimethylaminoazobenzene and 2-acetylaminofluorene		C																							
dimethylaminoazobenzene	(67)		C			C									C				C			C			
2-acetylaminofluorene	(73)			C		C									C				C	C		C			

From HSE (1980), ILO (1980), updated where applicable by ACGIH (1982), FRPTC (1983), ILO (1983), HSE (1985)

[a] *p* only
[b] *o*, *m* and *p*
[c] 2,4-isomer
C carcinogenic
CL carcinogenic substances to be used according to instructions of the Labour Inspectorate (Sweden)
H skin irritant
M ceiling value
S sensitizers

mg/m³ (IARC, 1982a). Although the diisocyanate of 2,4-diaminotoluene was detected in air samples from a polyurethane plant, the parent amine, a hydrolysis product of the diisocyanate, was not detected (IARC, 1978). The increased production of *p*-cresidine-based food dyes in recent years may have led to increased occupational exposure to *p*-cresidine (15) (IARC, 1982a).

4.2.2 Benzidines

Estimates of the number of people exposed to benzidines and derivative dyes during production and use are not definitely known. In 1973 it was reported that a total of 17 companies in the U.S.A. were using benzidine (45) and that 62 employees were potentially exposed. In the same report 18 companies in the U.S.A. had been confirmed to be using 3,3′-dichlorobenzidine (50) and a possible 250 employees were potentially exposed (IARC, 1982b). The NOHS estimated that 700 people were exposed occupationally to benzidine. However, this figure excluded exposure from the manufacture and use of dyes based on benzidine (IARC, 1982b), and the NTP (1983) reports that 2,200 workers are potentially exposed to benzidine in the U.S.A. Other NOHS estimates of numbers of people occupationally exposed to benzidine congeners in the U.S.A. include 200 to 3,3′-dimethoxybenzidine (48), 1,100 to 3,3′-dichlorobenzidine and 420 to 3,3′-dimethylbenzidine (46). NIOSH reported in 1978 that although the number of U.S.A. workers potentially exposed to large quantities of 3,3′-dimethylbenzidine was less than 100, as many as 200,000 may have been exposed to small quantities (NTP, 1983).

EPA (1979) reported the use of benzidine and congeners as analytical reagents in clinical laboratories. However, neither the size of the potentially exposed population nor the levels of exposure were known. IARC (1982b) reported that the result of a survey of forensic laboratories in the U.S.A. in 1974 showed that 54 of 276 laboratories were familiar with the benzidine-test for occult blood.

Between 1972 and 1974, NOHS estimated that approximately 79,000 workers in 63 different occupations were potentially exposed to benzidine-based dyes (NIOSH, 1980). These exposures occurred in the dye manufacturing, textile dyeing, printing, paper and leather industries. The numbers potentially exposed to the benzidine-based dyes, Direct Black 38 (76), Direct Brown 95 (78) and Direct Blue 6 (77), were estimated as approximately 13,000, 700 and 850 respectively. The greatest exposure was to Direct Red 1 where a potential exposure of 55,500 was reported (NIOSH, 1980). Dyes used in arts and crafts were not included in this study (see Section 4.1.2).

There are no occupational standards for benzidines or benzidine-based dyes. Airborne benzidine concentrations measured at different sites in a manufacturing plant ranged from <0.007 mg/m³ to 17.6 mg/m³ (IARC, 1982b). A Japanese study in 1970 of worker exposure to 3,3′-dichlorobenzidine in a pigment manufacturing plant showed that concentrations of 3,3′-dichlorobenzidine in air ranged between 2 and 25 μg/m³ during the charging of reaction vessels (IARC, 1982b).

The time-weighted average (TWA) environmental exposure levels of three workers exposed to the benzidine-based dyes Direct Black 38 and Direct Brown 95 were 0.69, 5.79 and 10.65 mg/m³ (average of three measurements) (NIOSH, 1980). In a paper-dyeing factory where approximately 1,700 kg of Direct Black 38 was used over a three-day period, 23 environmental samples were all less than 6 mg/m³ (range 0.17–5.10 mg total particulate matter/m³) (NIOSH, 1980). Since 1976 the

level of production of benzidine-based dyes has been greatly reduced in the U.S.A. This reduction has been accompanied by an increase in imported dyes which have been found to contain higher levels of free benzidine. It has been suggested that this may represent an increased risk to dye workers in the U.S.A. (EPA, 1979).

Some urinary levels of benzidines from workers exposed to different concentrations of airborne dyes are reported in Section 6.4.1.2.

4.2.3. 4,4′-Diaminodiphenylmethanes

In 1974 NOHS in the U.S.A. estimated that 33,000 workers were potentially exposed to 4,4′-methylenebis(2-chloroaniline) (NTP, 1983).

Exposure to 4,4′-methylenedianiline (53) in the vicinity of a mill used for blending an epoxy resin with the diamine, which was used as a hardener, was associated with a high incidence of toxic hepatitis (Section 6.5.4.1). Air concentrations of 4,4′-methylenedianiline of 0.8 mg/m^3 (0.1 ppm) were recorded during the early part of the illness episode, falling to 0.048 mg/m^3 (0.006 ppm) as control measures were introduced. Percutaneous absorption, however, was deemed to be the most likely route of exposure to 4,4′-methylenedianiline (McGill & Motto, 1974). At a facility where workers were blending or bagging a hardener containing 4,4′-methylenedianiline, air concentrations of 0.04−3 mg/m^3 (0.005−0.373 ppm) were recorded. Again, working conditions were such that considerable skin contamination was likely. Another study has found occupational exposure to 4,4′-methylenedianiline air concentrations of 0.24−29 mg/m^3 (0.03−3.8 ppm). The TWA exposure was 0.24−3.2 mg/m^3 (0.03−0.4 ppm) and highest concentrations occurred during the transfer of molten amine and grinding and packaging (ACGIH, 1980). IARC (1986) reported levels of up to 1.6 mg/m^3 (0.2 ppm) in the air of iron and steel foundries (where polymers were used as binders in moulding).

A combination of air monitoring, screening of urinary sediment by the Papanicolaou technique and urinary analysis for excreted 4,4′-methylenebis(2-chloroaniline) (54) has been used in an attempt to evaluate the extent of employee exposure at a plant manufacturing the amine. Fallout levels of 4,4′-methylenebis(2-chloroaniline) of 0.2−0.9 mg/m^2/d were recorded within a 30 m radius of the pelletizing and packaging facilities at a manufacturing plant. Within 2 m of the process, levels were as high as 1.2 mg/m^2/d but elsewhere in the plant fallout levels in excess of the detection limit (0.1 mg/m^2/d) were not found. Significant air concentrations (~70% as dust) were only found 1.2 m from the discharge end of the pelletizing unit. The maximum 8 h average concentration was 0.32 mg/m^3 as dust and 0.25 mg/m^3 as vapour (detection limit 0.01 mg/m^3). Using personal monitoring in the pelletizing and packaging section the maximum 8 h average concentration was 0.02 mg/m^3. The results of 4,4′-methylenebis(2-chloroaniline) urinary excretion analyses are summarized in Section 6.5.1.2. It was not possible to determine a relationship between urinary excretion and exposure dose because of the failure to demonstrate significant airborne contamination. However, atmospheric concentrations could not account for the observed urinary output and percutaneous absorption was thought to be the most likely route of exposure. The group of exposed individuals could be classified as 'excretors' and 'non-excretors' of 4,4′-methylenebis(2-chloroaniline). Excretors were found to be good biological monitors in that their urinary levels reflected changes in conditions at the plant such that during a period in which working

practices were improved the urinary levels recorded decreased (Linch et al., 1971). Atmospheric levels of 4,4'-methylenebis(2-chloroaniline) of 0.001-0.042 mg/m^3 were also reported in a factory producing rubber ski boots (HSE, 1983).

4.2.4 Naphthylamines

NIOSH has estimated that in the U.S.A. between 1972 and 1974 approximately 35,000 people were occupationally exposed to 1-naphthylamine (57) and 500 to 2-naphthylamine (58) and that 15,000 workers are potentially exposed to *N*-phenyl-2-naphthylamine (60) (Fishbein, 1980). Atmospheric concentrations of 2-naphthylamine (58) of 15 mg/m^3 were recorded at the open door of a mixer at a U.K. plant processing Nonox S, an antioxidant used in the past which was contaminated with naphthylamine isomers. A more recent survey of a plant processing rubber containing *N*-phenyl-2-naphthylamine and other related antioxidants contaminated with small amounts of 2-naphthylamine, reported no detectable atmospheric 2-naphthylamine (<0.7 μg/m^3). Exposure to an atmospheric concentration of 1 mg/m^3 for an 8 h shift would lead to inhalation of an estimated 10 mg/d (Nutt, 1983). From measurements of dusts in the air, levels of *N*-phenyl-2-naphthylamine of 10−100 mg/m^3 were found at one styrene-butadiene rubber plant, in an area where 50 kg bags of the amine were opened and mixed with oil, a procedure which took 15−20 minutes (Kummer & Tordoir, 1975).

4.2.5 Aminoazobenzenes

The NTP (1983) has estimated that approximately 2,500 workers in the U.S.A. are potentially exposed to *p*-dimethylaminoazobenzene (68).

Summary

The production volumes for aromatic amines are reported for a small number of countries only. The annual production of aniline (1) is at least several hundreds of millions of kilogrammes. Millions of kilogrammes of *o*-toluidine (2), 2,4-xylidine (5), *o*-anisidine (12) and *p*-anisidine (13), 2,4-diaminotoluene (34), 3,3'-dichlorobenzidine (50) and 4,4'-methylenedianiline (53) have also been reported to be produced annually. Several thousand kilogrammes of 4,4'-methylenebis(2-chloroaniline) (54) have been produced in the U.S.A. in recent years.

Most of the aromatic amines discussed herein do not occur naturally. They are relatively rapidly oxidized in air and water, but there are some compounds or decomposition products that have a relatively long residence time in soil. Aminoazobenzenes particularly, having relatively high lipophilicity, are more likely to bioaccumulate. Several aromatic amines have been reported to enter the food chain.

Aromatic amines have been detected in surface waters and foods. The general population may additionally be exposed via finished products in which aromatic amines have been used, such as textile dyes, rubber products, pesticides, food additives, and cosmetics. In the U.S.A. it has been estimated that more than 15 million

people in the general population may be exposed to phenylenediamines in consumer products despite their comparatively low production volumes (less than 10^6 kg/y). The only reports of numbers occupationally exposed to aromatic amines which have been found are those reported for the U.S.A., where several hundreds of thousands have been thought to be occupationally exposed to 2,4-diaminoanisole (36) (hairdressers and cosmetologists) and small quantities of 3,3′-dimethylbenzidine (46), and thousands may have been exposed to *o*-toluidine (2), *N,N*-dimethylaniline (9), a number of phenylenediamines, benzidine (45), 3,3′-dichlorobenzidine (50), 4,4′-methylenebis(2-chloroaniline) (54), and *p*-dimethylaminoazobenzene (68).

Part II. Toxicity

Sections 5 and 6 summarize the toxic effects of selected aromatic amines. The compounds are discussed according to the subdivisions in Part I (see Figure 1).

Section 5 reviews briefly the biochemical/mechanistic aspects of the predominant manifestations of aromatic amine toxicity. Allergic contact dermatitis is associated with exposure to several aromatic amines and because of its importance with respect to exposure to both the working and general populations, is reviewed in some detail in Section 6.1. Urinary bladder cancer has been associated for several decades with aromatic amine exposure and more recent evidence indicates that exposure to some aromatic amines may also be associated with cancers at other sites. Reports of cancers associated with specific compounds are reviewed in the relevant Sections 6.3 to 6.7. A brief summary of cancers believed to be associated with exposure to aromatic amines, but where the specific causative agent(s) has not been identified, is presented in Section 6.2. The toxicity of alternative aromatic amines introduced to replace known or suspected carcinogenic aromatic amines is reviewed in Section 6.8.

5. COMPARATIVE METABOLISM AND STRUCTURE-TOXICITY RELATIONSHIPS

5.1 Carcinogenicity

5.1.1 Biotransformation

Carcinogenic aromatic amines and aromatic amides require metabolic activation to form carcinogenic metabolites; the parent compounds are generally not direct-acting carcinogens. The metabolic activation of aromatic amines and aromatic amides has been reviewed by Bartsch (1981). For certain specific arylamines, ring epoxidation may play a role in carcinogenesis; however, *N*-hydroxylation is the major activation reaction. *N*-Hydroxylation alone is not sufficient to generate the ultimate carcinogenic species. Several pathways for metabolic activation have been identified. These include *N*-oxidation; conjugation of the *N*-hydroxy derivative to form a reactive ester intermediate such as the *N*-sulphate,*O*-acetyl or *O*-glucuronide or to form the *N*-glucuronide; peroxidase and free radical activation of arylhydroxamic acids; *N*-acetylation of arylamines; *N*-deacetylation of arylhydroxamic acids; reduction of nitro or azo groups in nitro compounds or azo dyes; and ring hydroxylation. The different routes of metabolic activation, and competition between them and

detoxification processes, probably in part explains the high specificity of aromatic amines for tumour induction in different organs in various species and strains of experimental animals and in humans.

N-Oxidation occurs mainly in the liver and may be catalysed by mixed function oxidases involving cytochrome P-450, as is the case with the *N*-hydroxylation of arylamides such as 2-acetyltyaminofluorene (72), and several primary amines, or by flavoprotein mixed function oxidases independent of cytochrome P-450, as is the case with the more basic amines such as some primary amines and secondary and tertiary amines (Kadlubar et al., 1976a; Bartsch, 1981; Kato et al., 1983; Gorrod & Damani, 1985). *N*-Hydroxylation may also occur in extra-hepatic tissue, for example, phenacetin (19) is readily hydroxylated *in vivo* and *in vitro* by both liver and kidney enzymes from most rodents and humans (Bartsch, 1981). *N*-Hydroxylation has been demonstrated to be subject to both intra- and inter-species variability; genetic differences in the levels and aromatic hydrocarbon inducibility of hydroxylating enzymes involved in 2-acetylaminofluorene and paracetamol (20) metabolism have been identified in mice (Thorgeirsson et al., 1975). In both the guinea pig and steppe lemming *C*-hydroxylation predominates over *N*-hydroxylation in arylamine metabolism. This finding is consistent with the observed absence of arylamine-induced carcinogenicity in these species (Williams & Weisburger, 1986).

O-Sulphonation catalysed by 3′-phosphoadenosine-5′-phosphosulphate (PAPS)-dependent rat liver cytosol sulphotransferases, to form an *N*-sulphate, has been demonstrated for *N*-hydroxy-2-acetylaminofluorene (De Baun et al., 1970), some hydroxylaminoazobenzenes including *N*-hydroxy-*p*-methylaminoazobenzene, and the *N*-hydroxyderivatives of 1- and 2-naphthylamine (Kadlubar et al., 1976b). The hepatocarcinogenicity of 2-acetylaminofluorene in the rat correlates well with the *in vivo* activity of *N*-hydroxy-2-acetylaminofluorene sulphotransferase. The sulphuric acid ester is considered to be the major ultimate hepatocarcinogenic metabolite in this system, interacting with liver macromolecules via an arylamidonium ion (De Baun et al., 1970). Just as different hepatic enzymes were found to catalyse their *N*-oxidation, the *O*-sulphonation of *N*-hydroxy-2-acetylaminofluorene and *N*-hydroxy-*p*-methylaminoazobenzene is apparently mediated by different hepatic sulphotransferases. Though the greatest activity was found in the male rat liver, hepatic sulphotransferase activity for *N*-hydroxy-*p*-methylaminoazobenzene was also found in some other rodent species. Low levels of sulphotransferase activity were also found in the male rat kidney and small intestine cytosol (Kadlubar et al., 1976b). The experimental observations are consistent with the fact that *p*-methylaminoazobenzene (68) and related dyes are only strongly carcinogenic in male rat liver. The observation that the hepatocarcinogenicity of 3′-methyl-4-dimethylaminoazobenzene (69) is enhanced in the rat by dietary administration of high levels of sodium sulphate is also consistent with the importance of metabolic activation via *O*-sulphonation (Blunck & Crowther, 1975). The demonstrated low hepatocarcinogenicity of 1-(57) and 2-naphthylamine (58) and 4-aminobiphenyl (72) is possibly due to the low rate of *O*-sulphonation of their *N*-hydroxymetabolites, in rat liver, relative to *N*-hydroxy-*p*-methylaminoazobenzene.

O-Acetylation to form *N*-acetoxy compounds is another pathway for the activation of the *N*-hydroxy derivative of *N*-oxidized aromatic amines and amides. *N,O*-Acyltransferases, which are found in a wide variety of tissues in the rat and other species, including target tissues devoid of sulphotransferase activity, catalyse the transfer of the *N*-acetyl group from *N*-hydroxy-2-acetylaminofluorene to the oxygen atom of the corresponding arylhydroxylamine (King & Olive, 1975). Similarly, the

in vitro binding of *N,N'*-diacetyl-*N*-hydroxybenzidine to RNA in the presence of liver cytosol from various rodent species has been shown to be catalysed by *N,O*-acyltransferase (Morton et al., 1979).

The *N*-hydroxy derivatives of both carcinogenic aromatic amines and amides may be activated by *O*-glucuronidation. The hydroxylamines may also be activated by *N*-glucuronidation (Bartsch, 1981). The observations that glucuronic acid conjugates of 1- and 2-naphthylamine and 4-aminobiphenyl are excreted in the urine of dogs, and that the *N*-glucuronide of the related hydroxylamine can be synthesized in a UDPG-dependent reaction in the presence of liver fraction from several species *in vitro* suggest that these arylamines may be *N*-hydroxylated and glucuronidated in the liver, then transported to the urinary bladder, where hydrolysis in the normally acidic urine of dogs and humans regenerates the direct-acting carcinogenic *N*-hydroxylamine, which may interact with cellular nucleophiles via a highly reactive nitrenium ion (Miller, 1978). This is the currently accepted route of metabolic activation for arylamine bladder carcinogens.

Arylhydroxamic acids may undergo both enzymatic and non-enzymatic 1 electron oxidation *in vitro* to generate a free nitroxide radical which dismutates to yield the corresponding *N*-acetoxy-*N*-acetylaminoarene and nitrosoarene. Such a reaction has been demonstrated for the *N*-hydroxy-*N*-acetyl-derivatives of several aromatic amines including 2-aminofluorene, 1- and 2-naphthylamine and 4-aminobiphenyl, which readily undergo 1 electron oxidation in the presence of $Fe(CN)_6^{3-}$ or Ag_2O, or horseradish peroxidase and H_2O_2 at pH 7. One electron oxidation of *N*-hydroxy-2-acetylaminofluorene has also been shown for lactoperoxidase and human myeloperoxidase. An apparently inverse relationship between the stability of the free nitroxide radical and carcinogenesis by arylhydroxamic acids has been observed (Bartsch et al., 1972). Recent evidence indicates that a prostoglandin H synthase-mediated peroxidative metabolism may be involved in the activation of arylamine-induced bladder cancer (Yamazoe et al., 1985; Wise et al., 1986; Zenser et al., 1986).

N-Acetylation *in vivo* and *in vitro* has been demonstrated for a number of aromatic amines. Mono- and diacetyl-derivatives of benzidine (45) and its congeners have been observed in the urine of exposed humans and experimental animals (Haley, 1982). *N*-Acetylation may modulate susceptibility to aromatic amines in terms of both species and tissue susceptibility. For example, both liver and bladder tumours may be induced in dogs following administration of *N*-acetylarylamines. However, dogs are incapable of acetylating aromatic amines and only bladder cancers can be induced following administration of the related parent amines. Aminofluorene, 1- and 2-naphthylamine, benzidine and 4,4'-methylenebis(2-chloroaniline) (54) have all been found to be acetylated by the same genetically determined polymorphic acetyltransferase as isoniazid and sulphamethazine in human and rabbit liver fractions (Glowinski et al., 1978). The occurrence of a genetic polymorphism for *N*-acetylation suggests that susceptibility to arylamine-induced carcinogenesis may also be genetically determined. Since the parent amine will be available as a substrate for *N*-oxidation for a longer time in individuals displaying a 'slow acetylator' phenotype, they may produce more hydroxylamines which may be glucuronidated and transported to the urinary bladder. Slow acetylators therefore might be expected to be at a higher risk from aromatic amine-induced bladder cancer. Lower et al. (1979) have demonstrated a relative risk for bladder cancer of 1.74 for slow acetylator phenotypes in an urban population where aromatic amines might be expected to play a role in disease etiology, but no excess risk for slow acetylators in a rural population where such a role is less likely. Cartwright et al., (1982) reported an excess of slow

acetylators in a group of bladder cancer patients with a history of employment in the dyestuffs industry. *N*-acetylation may therefore be a detoxification pathway with respect to bladder cancer, which would explain why dogs are such a good model for bladder cancer (Lower, 1982). Conversely, *N*-acetylation followed by oxidation appears to predominate in species which develop primarily liver tumours (Beland et al., 1983).

N-Deacetylation may also be an activation pathway for arylacetamides and may be subject to genetically determined variation (Wolf et al., 1980). Following administration of various carcinogenic arylacetamides to dogs, susceptibility to bladder carcinogenesis is correlated with the specificity of arylacetamide liver *N*-deacetylase enzymes, indicating that deacetylation may be required for bladder carcinogenesis in dogs (Lower & Bryan, 1976).

Reductive cleavage of azo double bonds may be involved in both activation and detoxification of azo dyes, depending on whether or not the amines produced by reduction may be further metabolized to carcinogenic species (Bartsch, 1981). Azo reduction is a major detoxification pathway for *p*-dimethylaminoazobenzene (67), for example, which after feeding to rats is metabolized and excreted in the urine as *N*-acetyl-*p*-aminophenol and *N*,*N*′-diacetyl-*p*-phenylenediamine (Miller & Miller,1983). Metabolism of azo dyes, however, does not necessarily include reduction of the azo group (Walker, 1970). Reductive cleavage may also activate pigments and dyes based on benzidine and its congeners by conversion to the parent amine. Several species reductively cleave benzidine dyes to benzidine (Haley, 1982). Enzymes responsible for reducing azo bonds are found mainly in the liver in mammalian species, although azo reductase activity has been observed in other tissues (Walker,1970). Azo reduction may also occur in the stomach and small intestine before absorption due to the activity of gut microflora (Walker, 1970; Chung, 1983). Water soluble azo dyes are more likely to be degraded by intestinal micro-organisms whereas water insoluble azo dyes are metabolized by liver enzymes (Chung, 1983). It has been suggested that intestinal azo reducing systems are generally more active and non-specific than hepatic azo reductases (Walker, 1970). Moreover, there is some evidence that hepatic azo reductases may play only a minor role in some species in the azo reduction of dyes derived from benzidine and its congeners (Chung, 1983).

Although ring hydroxylation of aromatic amines has generally been considered to decrease or remove carcinogenic activity (Arcos & Argus, 1968), recent evidence suggests that in the case of 2-Naphthylamine, *ortho*-hydroxylation may be an important activation pathway (Yamazoe et al., 1985). It has been proposed that *o*- and *p*-ring hydroxylation of aromatic amines may occur via rearrangement from the *N*-hydroxy-metabolites *in vivo* (Arcos & Argus, 1974).

In conclusion, differences in species and tissue susceptibility to various aromatic amines may in part be due to different routes of metabolic activation, some of which may be subject to genetic variability. Some metabolic pathways may activate carcinogenic aromatic amines in some conditions and detoxify them in others.

5.1.2 Structure-activity relationships

The carcinogenicity of aromatic amines in terms of possible qualitative structure-activity relationships has been reviewed (for example, Arcos & Argus,1968; Radomski, 1979). Radomski compared the results obtained from continuous oral administration to dogs of a number of aromatic amines. Some of the discussion and

conclusions are summarized herein. Compounds with conjugating benzene rings such as 4-aminobiphenyl (72), naphthylamines and benzidine (45) do not significantly induce liver toxicity or carcinogenicity whereas those with a carbon or nitrogen bridge between 2 benzene rings such as diaminodiphenylmethanes may. In general, arylacetamides induce liver cancer whereas the amines induce bladder cancer. Differences in metabolism probably explain the latter observation. Whereas acetamides are *N*-hydroxylated in the liver to form a hydroxamic acid which may undergo transacetylation or esterification to yield a reactive species capable of interacting with cellular constituents, the resulting hydroxylamine from hydroxylation of arylamines is not reactive at tissue pH. Conversely, glucuronidation of hydroxamic acids forms a stable conjugate which does not hydrolyze in the bladder whereas in dogs, for example, the corresponding glucuronide of an arylhydroxylamine following transport to the bladder may hydrolyze to regenerate the reactive hydroxylamine (Section 5.1.1).

It has been proposed that in order to be carcinogenic an aromatic amine must have an uninterrupted conjugated system with the amino group attached to the carbon at the terminal *para*-position. In general, aromatic amines with a resonance form such that the carbon *para* to the amino group is conjugated with another aromatic ring are carcinogens, whereas those for which this is not the case (for example, 1-naphthylamine (57)) or for which conjugation is interrupted by a single carbon bridge are not bladder carcinogens. This is not always the case, however. For example, whereas 4,4′-methylenebis(2-methylaniline) (55), as might be expected from the *para* principle, is not a bladder carcinogen in dogs, 4,4′-methylenebis(2-chloroaniline) (54) is. Radomski (1979) comments that the importance of ring conjugation with respect to carcinogenic activity may be due to resonance stabilization of the ultimate reactive species. Both a nitrenium ion and a free radical have been proposed as the ultimate reactive species from hydroxylamines. The nitrenium ion would be expected to be stabilized by electron-releasing substituents and the free radical by electron-withdrawing substituents. The observation in dogs that an electron-releasing substituent such as $-NH_2$ decreases the potency of 4-aminobiphenyl (72) (i.e. benzidine (45) is less active) whereas dichlorobenzidine (50) (electron-withdrawing substituents) is a more potent bladder carcinogen than benzidine may be indicative of a free radical intermediate for biphenyls in this species (Radomski, 1979). Other substituent effects may include steric interactions which may, for example, distort planarity and therefore lower resonance. Also, some substituents may be capable of, or enhance, non-covalent interactions with critical cellular sites and, by binding the molecule in such a way, increase carcinogenic activity (Arcos & Argus, 1968). Methyl substitution raises the conjugation power of the aniline molecule and appears to increase carcinogenic activity (Arcos & Argus, 1968). Milman & Peterson (1984) in a review of carcinogenicity studies in experimental animals with some phenylenediamines concluded that, in general, for the small number of compounds studied, *p*-phenylenediamines and related compounds were not carcinogenic and ring substituted *m*-phenylenediamines and related compounds were carcinogenic.

The effects of some 4′-substituents on the carcinogenicity of 4-aminobiphenyls have been studied in rats. The 4′-fluoro-derivative is an active carcinogen whereas 4′-chloro- and 4′-bromo-derivatives have no activity and 4′-methyl-derivative has borderline activity. Similar trends are apparent for *p*-dimethylaminoazobenzenes where 4′-fluoro-derivative is an active hepatocarcinogen after oral dosing in rats, 4′-chloro- and 4′-methyl-derivatives are weak carcinogens and 4′-bromo-derivative

is inactive. Other substituents, however, for example amino and nitro groups do not have similar parallel effects (Arcos & Argus, 1968).

As with anilines, the carcinogenic activity of diaminodiphenylmethanes also appears to be enhanced by ring methylation. Methylenebis(2-methylaniline) (55) is, for example, a more active carcinogen p.o. in rats than 4,4′-methylenedianiline (53) (Arcos & Argus, 1968).

The structural requirements for hepatocarcinogenicity of *p*-dimethylaminoazobenzenes in the rat have been described by Fishbein (1977). Apart from the effects of 4′-substitution, at least one *N*-alkyl group is required for the hepatocarcinogenicity of *p*-aminoazobenzene dyes (Arcos & Argus, 1968), and *N*-dealkylation is therefore regarded as a detoxification pathway for *N*-alkylated *p*-aminoazobenzenes (Arcos & Argus, 1974). Bebawi et al. (1970) studied the carcinogenicity of *p*-dimethylaminoazobenzenes administered orally to rats and reported that the relative carcinogenic activity of 3′,4′-disubstituted dimethylaminoazobenzenes could be predicted from the theoretical summation of the relative activities of each of the substituents separately, i.e. the effect of substitution was additive. Structure-activity relationships for mutagenicity/carcinogenicity for a number of aminoazobenzenes have been reviewed by Combes & Haveland-Smith (1982).

A preliminary paper by Lavenhar & Maczka (1985) describes the use of quantitative structure-activity relationships in the carcinogenic risk estimation of a number of anilines and phenylenediamines tested in the NCI Bioassay Program.

A better understanding of structure-activity relationships would be of assistance in predicting the toxicity of new or untested aromatic amines and could be a contributory factor in the selection of new chemicals introduced to replace known carcinogenic aromatic amines withdrawn from use.

5.1.3 Nucleic acid and protein adducts

Early attempts to determine the structures of cellular constituent adducts with carcinogenic aromatic amines included the work by the Millers and co-workers on liver protein-bound derivatives of *p*-dimethylaminoazobenzene (67) (for review see Miller & Miller, 1983). Since then several reports have identified nucleic acid and protein adducts with aromatic amines or their proposed metabolites *in vitro* and *in vivo*.

Following studies with some aminobiphenyls, naphthylamines and *p*-methylaminoazobenzenes or their *N*-hydroxy- or *N*-acetyl-derivatives, the *C*8-deoxyguanosine adduct has been identified frequently both *in vitro* and *in vivo* in hepatic and bladder epithelium DNA. Other adducts reported include *C*8- and N^6-substituted deoxyadenosine and N^2- and O^6-substituted deoxyguanosine. In the case of the aminobiphenyls and *p*-methylaminoazobenzenes the DNA adducts have been identified bound to the aromatic amino nitrogen, whereas for the naphthylamines, DNA adducts bound to either the amino nitrogen or to the aromatic ring *ortho* to the amino group have been reported (Kadlubar et al., 1976b; Miller, 1978; Bartsch, 1981; Beland et al., 1983; Martin et al., 1983; Yamazoe et al., 1985, 1986).

Aromatic amine-protein adducts have been characterized *in vitro* and *in vivo*. For example, hepatic adducts isolated *in vivo* from rats administered *p*-methylaminoazobenzene include cysteine-, tyrosine- and methionine-bound derivatives (Miller, 1978; Bartsch, 1981). Protein adducts, or their degradation products, from *p*-methylaminoazobenzene (67) or its *N*-benzoyloxyester or *N*-hydroxy-derivatives,

have been identified *in vitro* and *in vivo* bound at either the amino nitrogen or to the aromatic ring at the 3-position (*ortho* to the amino group). 3-Methylmercapto-derivatives have been isolated frequently as degradation products from 3-methion-*S*-yl-*p*-methylaminoazobenzene protein bound residues (Arcos & Argus, 1968; Kadlubar et al., 1976b; Miller, 1978; Bartsch, 1981; Miller & Miller, 1983). Similarly, a 1-mercapturic acid derivative has been detected in the urine of animals administered 2-naphthylamine (58). Its formation has been proposed to be via *N*-hydroxylation and the formation of an *N*-sulphhydryl compound, paralleling the proposed metabolic transformation of *N*-hydroxy-2-naphthylamine to *o*-hydroxy-2-naphthylamine (Arcos & Argus, 1968). Both albumin and haemoglobin adducts have been identified following administration of 4-aminobiphenyl to rats (Skipper et al., 1984). A benzidine-albumin complex was identified in the serum of workers exposed to Direct dyestuffs. Such a complex was not found in workers exposed to non-Direct dyes (IARC, 1982b).

5.2 Methaemoglobinaemia: biotransformation

Ferrihaemoglobin is formed by the 1 electron oxidation of the ferrous ion of haemoglobin to the ferric ion. Whereas the ferrous ion of haemoglobin can reversibly bind with molecular oxygen to form oxyhaemoglobin, the ferric ion cannot and when ferrihaemoglobin levels are sufficiently in excess of normal, methaemoglobinaemia, a condition characterized by cyanosis and hypoxia, may result. Ferrohaemoglobin is autoxidized to ferrihaemoglobin but a normal red cell concentration of less than 1% is usually maintained by reducing enzymes such as NADH diaphorase and cytochrome. Newborn infants are particularly susceptible to chemically induced methaemoglobinaemia since they generally have low levels of NADH diaphorase. Hereditary disorders, such as a deficiency in the reductase content of red cells, or the presence of certain abnormal haemoglobins also predispose to methaemoglobinaemia (Kiese, 1974; Beard & Noe, 1981).

There are two principal metabolic pathways responsible for the induction of methaemoglobinaemia by aromatic amines; one involves aminophenols and the other hydroxylamines. The evidence for both pathways have been reviewed by Kiese (1974) and only a brief summary will be presented here.

p-Hydroxylation of aniline *in vitro* is catalysed by cytochrome P-450 and other haemoproteins, in a reaction which appears to be mediated by hydrogen peroxide, superoxide anions and hydroxyl radicals (Ingelman-Sundberg & Ekström, 1982). Oxygen is required for aminophenol activity *in vitro*. The aminophenol oxidizes ferrohaemoglobin to ferrihaemoglobin via a quinoneimine intermediate formed either by the autoxidation of aminophenol or from the reaction between oxyhaemoglobin and aminophenol. The aminophenol produces several equivalents of ferrihaemoglobin in blood or haemoglobin solution *in vitro*. *N*-alkyl-*p*-aminophenols produce ferrihaemoglobin more rapidly than *p*-aminophenol, *o*-aminophenol also reacts more rapidly than the *p*-analogue and again *N*-methylation increases the reaction rate. Ring substitution may also affect the reaction rate; ring halogenation increases activity. *In vivo*, additional routes of elimination, apart from ferrihaemoglobin formation, for example glucuronide or sulphate conjugation, modify the yield of ferrihaemoglobin. In humans, the maximum concentration of ferrihaemoglobin formed was proportional to the dose for several aminophenols administered i.v. for doses oxidizing not more than 30% haemoglobin.

Oxygen is also required for the hydroxylamine route to ferrihaemoglobin formation *in vitro*. Nitrosobenzene and ferrihaemoglobin are produced from the reaction between haemoglobin and phenylhydroxylamine. The mode of action of arylhydroxylamines has not been fully elucidated but is discussed in detail by Kiese (1974). In the presence of hydrogen sulphide and oxygen phenylhydroxylamine also produces sulphaemoglobin, a green derivative of haemoglobin. In red blood cells *in vitro*, nitrosobenzene formed from the reaction between oxyhaemoglobin and phenylhydroxylamine is reduced to phenylhydroxylamine thereby establishing a cycle in which one molecule of phenylhydroxylamine transforms many equivalents of ferrohaemoglobin to ferrihaemoglobin. Experiments indicate that a NADPH diaphorase and the pentose phosphate pathway are major sources of electrons for the reduction of nitrosobenzene. *In vivo* the amount of ferrihaemoglobin formation may be modified by the loss of arylhydroxylamines via other routes which may vary with species and substance.

The major active methaemoglobin-inducing metabolite from an aromatic amine may vary from species to species and substance to substance, for example, an aminophenol is probably the active metabolite from *m*-phenylenediamine (32) in the dog, whereas phenylhydroxylamine is the metabolite which produces the most ferrihaemoglobin from aniline (1) in the same species (Kiese, 1974). The weight of evidence, however, indicates that *N*-hydroxylation is the primary route to formation of methaemoglobin-inducing agents from aromatic amines (Radomski, 1979).

Induction of methaemoglobinaemia by several aromatic amines has been demonstrated in experimental animals and has been extensively reviewed by Kiese (1974). Aniline and substituted anilines, including some phenylenediamines, naphthylamines and aminoazobenzenes, have all been shown to cause methaemoglobinaemia in one or more species. Methaemoglobinaemia is induced in a dose-related response to administration of aniline in several species. In dogs, for example, the maximum level of methaemoglobin in the blood is proportional to the log of the aniline dose and is independent of the route of administration. Table 8 indicates some compounds which have been demonstrated to induce methaemoglobin formation in animals.

5.3 Allergic contact dermatitis: structure-activity relationships

Several aromatic amines cause allergic contact dermatitis (see Section 6.1). A primary amine group in the molecule significantly influences the sensitizing properties of an aromatic compound, irrespective both of whether or not there are other substituents on the ring, or the position of the amino function (i.e. *ortho*-, *meta*-, or *para*-) in relation to any other ring substituents (Kleniewska, 1975). Secondary and tertiary amines and their acylated derivatives do not seem to have sensitizing properties and do not cross-sensitize with the primary aromatic amines.

In addition to the amino group, the other substituents in the aromatic ring may have an effect on the allergenicity. The substituents can be divided into electron donors and electron acceptors (Hauptmann et al., 1976). It has been shown that electron donor substituents increase the electron density in the aromatic ring as well as in the nitrogen of the amino group on the ring. The basicity of the amino group is increased and as a consequence an increase in the reactivity of the amine as a nucleophilic agent is obtained (Malkowski et al., 1983). Thus aromatic amines with electron donor substituents in the ring can be expected to exhibit an enhanced allergenicity compared with unsubstituted aniline. Conversely, electron acceptor

Table 8 Methaemoglobinaemia in experimental animals[a]

Compound	Compound No.	Species					
		Cat	Dog	Rat	Rabbit	Mouse	Guinea pig
Aniline	(1)	+	+	+	+	+	
o-Toluidine	(2)	+	+	+[b]		+[b]	
m-Toluidine	(3)	+	+				
p-Toluidine	(4)	+	+				
2,4-Xylidine	(5)	+		+			
2,5-Xylidine	(6)	+		+			
2,4,5-Trimethylaniline	(10)			+			
2,4,6-Trimethylaniline	(11)			+			
o-Anisidine	(12)	+					
p-Anisidine	(13)	+					
2,4,6-Trichloroaniline	(29)	+					
m-Phenylenediamine	(32)	+	+				
p-Phenylenediamine	(33)		+	+			
2,4-Diaminotoluene	(34)	+	+	+[c]	+[c]		+[c]
1-Naphthylamine	(57)		+				
2-Naphthylamine	(58)	+	+				
p-Aminoazobenzene	(65)			+			
p-Dimethylaminoazobenzene	(67)			+			

From:
[a] Kiese (1974) unless otherwise indicated
[b] IARC (1982a)
[c] IARC (1978)

substituents introduced into the ring of aromatic amines decrease the electron density in the ring and lower the effective negative charge in the amino group, decreasing its basicity and consequently its reactivity. Therefore, aromatic amines with such substituents should exhibit lower allergenicity as compared to aniline. Experiments fully confirm the validity of this hypothesis. Amino, hydroxyl, and methyl groups introduced into the aniline ring enhance its allergenicity, whereas the nitro, sulphonic, and carboxyl groups attenuate this activity (Kleniewska & Maibach, 1980).

The biological activity of the compound is affected by the position of the substituent in the ring relative to the amino group. Data concerning cross-allergy between *p*-phenylenediamine and a series of *ortho*-, *meta*-, and *para*-aromatic amines suggest that the *ortho* position is exceptional in so far as the introduction of a substituent, even an electron-donor, into this position decreases allergenicity. An electron acceptor substituent in this position greatly depresses allergenicity of an aromatic amine, e.g. the nitro group in *o*-nitroaniline (21) (Kleniewska, 1975).

6. CLINICAL STUDIES AND SHORT-TERM TESTS

6.1 Human dermatoses following exposure to aromatic amines

Because of their alkaline nature, certain aromatic amines, particularly the primary amines, constitute a potential hazard with respect to contact dermatitis.

The principal dermatological response to aromatic amines is allergic contact dermatitis, although irritant contact dermatitis may contribute to some of the effects observed. Although contact urticaria (type I allergy), which refers to a wheal and flare response elicited within a few minutes to an hour after intact skin is exposed to certain rapidly absorbable agents (Odom & Maibach, 1977) can occur, the major response to aromatic amines is contact allergy (type IV allergy), which refers to a delayed hypersensitivity. Contact allergy involves an immunological reaction in the skin. No visible alteration occurs following initial contact with the allergen (sensitizer), but the cellular changes which occur mean that the skin will react differently on subsequent exposure to the allergen and dermatitis can develop.

Contact sensitivity to aromatic amines has been recognized for many years. Marked differences in the allergenicity of aromatic amino compounds have been found from clinical experiences and animal assays. *p*-Phenylenediamine (33) has been established to be a potent sensitizer, whereas certain local anaesthetics, sulphonamides and *p*-aminobenzoic acid (17) have been found to be weaker sensitizers. Although the results from many studies in experimental animals have contributed extensively to knowledge about allergic contact dermatitis induced by aromatic amines, these are not discussed at length in this document which deals mainly with effects observed in humans.

An apparent cross-reaction between, or simultaneous sensitization by, different aromatic amines frequently occurs and constitutes an important cause of continuation, exacerbation, and recurrence of occupational dermatitis under conditions not connected with work. Positive tests with a number of aromatic amines are often obtained in the same patient. This may be due to cross-reactions between different aromatic amines. However, primary hypersensitivity to several of the chemicals must also be considered. This may arise from separate exposures to several aromatic amines or due to impurities in chemicals, simultaneous sensitization to several compounds in a mixture may be a common occurrence. Hereafter the term multiple hypersensitivity is used to describe any or all of these possibilities. The problems

associated with the identification of true cross-sensitization reactions have been discussed recently (Benezra & Maibach, 1984; Fregert, 1985).

It has been suggested that multiple hypersensitivity may be mediated by the formation of similar quinone or quinoneimine intermediates which would then act as the actual allergenic species (Mayer, 1954). However, several *meta*-substituted aromatic amines as well as *ortho*- and *para*- compounds are involved in apparent group allergies and *meta*-substituted aromatic amines cannot be transformed into simple quinones (Kleniewska, 1975).

p-Phenylenediamine is the most studied of the aromatic amines which cause allergic contact dermatitis or multiple hypersensitivity, and many clinical investigations have arisen from an initial observation of a response to *p*-phenylenediamine. Multiple hypersensitivity has been observed involving reactions to *p*-phenylenediamine and some other anilines and phenylenediamines, and some other compounds within the benzidine,4,4′-diaminodiphenylmethane, or aminoazobenzene classes of aromatic amines.

Although the term '*para*-group' allergy has been used in the literature to describe multiple hypersensitivity to aromatic amines and related compounds, in fact it is not a requirement that the amino group should be *para* to another substituent on the benzene ring for the effect to be observed and the term '*para*-group' is not used in this document. *Ortho*- and *meta*-substituted aromatic amines may also be involved in group allergies. Thus, Kleniewska (1975) reported multiple hypersensitivity to a number of *ortho*-, *meta*- and *para*-isomers of aromatic amines in people sensitive to *p*-phenylenediamine. Of the *p*-phenylenediamine sensitive patients, 60% were also sensitive to *o*-phenylenediamine (31), 70% to *m*-phenylenediamine (32), 64% to *p*-toluidine (4), 25% to *o*-toluidine (2), 63% to *m*-toluidine (3), 37% to *p*-aminophenol, 35% to *o*-aminophenol, 38% to *m*-aminophenol, 14% to *p*-nitroaniline (23), 18% to *m*-nitroaniline (22), 8% to 2-chloroaniline (26), 53% to 3-chloroaniline, 68% to 2,3-diaminotoluene, and 68% to 2,4-diaminotoluene. Positive reactions were obtained only with primary aromatic amines and were independent of the position of the amino group in the ring – *ortho*, *meta* or *para*. Aromatic nitro and hydroxyl compounds as well as secondary and tertiary amines did not cause multiple hypersensitivity.

In another investigation of 100 patients with positive test reactions to *p*-phenylenediamine, benzidine (45), and/or benzocaine, who were tested with 25 aromatic amines, azobenzene (64) and 2 azodyes, four were positive only to *p*-phenylenediamine, four to benzidine only and three to benzocaine only, while the remaining patients showed multiple hypersensitivity (Rudzki, 1975). There does not seem to be any identical sensitization pattern in that only two of the 100 patients reacted identically to all the aromatic amines, azobenzene, and azodyes. According to Baer (1954), in general the stronger the hypersensitivity to the primary allergen, the greater the tendency to multiple hypersensitivity. No good method of measuring the intensity of sensitization is available however.

In patients positive to many amines, it is sometimes very difficult to detect the primary sensitizer. In many patients, however, the sensitizing agent is known. In, for example, many of the industrial rubber products, the antioxidant *N*-*i*-propyl-*N*′-phenyl-*p*-phenylenediamine (39) has been found to be the primary sensitizer.

The occurrence of possible cross-reactions between *p*-phenylenediamine and rubber antioxidants which are derivatives of *p*-phenylenediamine is unclear. Individuals sensitive to *N*-*i*-propyl-*N*′-phenyl-*p*-phenylenediamine and also other related rubber antioxidants are often, but not always, also sensitive to *p*-phenylenediamine. In 42

cases of occupational *N-i*-propyl-*N'*-phenyl-*p*-phenylenediamine sensitivity, mostly in the tyre manufacturing industry and in automobile transport (Table 9), 15/40 of the patients were also positive to *p*-phenylenediamine, although the test reactions to *p*-phenylenediamine were considerably weaker than to *N-i*-propyl-*N'*-phenyl-*p*-phenylenediamine, and 8/8 of the subjects senstive to *N-i*-propyl-*N'*-phenyl-*p*-phenylenediamine were also positive to *N*-phenyl-*N'*-cyclohexyl-*p*-phenylenediamine, another amine antioxidant (Hervé-Bazin et al., 1977). Similarly, in another investigation among 31 patients positive to *p*-phenylenediamine, 16 were also positive to *N-i*-propyl-*N'*-phenyl-*p*-phenylenediamine. Positive tests with the antioxidants were somewhat more frequent among subjects who reacted to several amines. It seems, however, that in patients sensitive to numerous amines, a positive response to *N-i*-propyl-*N'*-phenyl-*p*-phenylenediamine is less relevant (Rudzki et al., 1976). Moreover, the patch test sensitizations to *N-i*-propyl-*N'*-phenyl-*p*-phenylenediamine were found to include sensitivity to the closely related dye *N*-phenyl-*p*-phenylenediamine (38) also (Schonning & Hjorth, 1968).

Multiple hypersensitivity to aromatic amines may occur not only to chemicals in the rubber industry but also to allergens used in film laboratories and by photographers, hairdressers, etc. Positive tests to several aromatic amines were observed in 3/5 photographers sensitized to *N,N'*-diethylphenylenediamine and/or hydroquinone and in 2 hairdressers with probable primary sensitization to *p*-phenylenediamine (Rudzki, 1976). Similarly, simultaneous positive reactions to 4,4'-methylenedianiline (53) and other aromatic amines such as *p*-phenylenediamine, benzidine and azo-compounds have been reported (Agrup & Fregert, 1969). Primary sensitization to *m*-phenylenediamine used as a hardener has been described by Rudzki et al. (1976) in subjects working with epoxy resins. One of the investigated patients gave a postive response on testing not only to *m*-phenylenediamine but to other aromatic amines also (Rudzki & Krajewska, 1974) (Table 9 and Section 6.3.4.2).

The dyes used in textiles are usually designated as "aniline" colours. The term "aniline" refers to all types of synthetic colours whether or not they are derived from aniline. Many azo dyes may also be included in this category (see Section 6.7.4.2). Although many hundreds of dyes are used in different products (textiles, leather, hair dyes, ball-point pens, etc.), few cause sensitization. The dyes which sensitize most are derivatives of *p*-phenylenediamine, aniline, benzidine and azobenzene.

Although textiles dyed with synthetic dyestuffs have been worn on a vast scale for nearly a century with much of these dyed fabrics in direct contact with the skin, the instances of dermatitis attributed to dyes in wearing apparel is remarkably small. Reports for various types of clothes have been summarized by Cronin (1968). From 1970 to 1976 only 21 women and 26 men with dermatitis from clothing were seen at the Contact Clinic, St John's Hospital in London (Cronin, 1980). Dermatitis in the textile industry has been reviewed in a monograph by Cywie et al. (1977). The authors emphasize that the vast number of dyes, their many trade names, and their impurity make accurate diagnosis difficult.

Although *p*-phenylenediamine, with the exception of fur dyeing, is not used in clothing, allergic sensitization to *p*-phenylenediamine may play a significant role in the production of dermatitis due to "aniline" dyes. The "aniline" dyes are weak sensitizers and apparently are rarely the cause of primary sensitization. In most cases, when dermatitis is associated with the anilines and azodyes an existing sensitization against *p*-phenylenediamine related substances may have been reactivated. Thus, it has been suggested that the primary sensitization caused by *p*-phenylenediamine,

Table 9 Allergic contact dermatitis

Compound	Compound No.	Exposure/Response	Reference
Phenylenediamines		*1 Prevalence tests* (i.e. tests not carried out for diagnostic purposes)	
m-phenylenediamine	(32)	scratch test production workers aged 30–50 y, 5–10 y exposure 8% incidence of positive response (see also Table 10)	Orlov (1974)[a]
p-phenylenediamine	(33)	normal population (male 21–50 y) patch test (predictive) 53% (47/88) incidence of positive response to 1% (induction and challenge) in petrolatum	Marzulli & Maibach (1974)
		normal population (male) maximization test (predictive) 45% (15/34) incidence of positive response to 2% (induction and challenge) aqueous solution	Epstein & Taylor (1979)
		2 Cases and diagnostic tests	
		2.1 Unspecified exposure	
p-phenylenediamine	(33)	1958–1961, 10.6% of 2903 patients suffering from eczema gave positive response to 2% (aq) *p*-phenylenediamine	Modée & Skog (1962)[a]
		6% of 691 patients with allergic dermatoses gave positive patch test with *p*-phenylenediamine	Korossy et al. (1969)[a]
		237/4825 (4.9%) and 97/1200 (8%) (2 studies) eczema patients showed positive skin test with *p*-phenylenediamine (1%)	Rudner et al. (1973)

Table 9/contd

Compound	Compound No.	Exposure/Response	Reference
Phenylenediamines contd		*2.2 Rubber chemicals*	
N-i-propyl-*N'*-phenyl-*p*-phenylenediamine	(39)	lichenoid dermatitis following occupational handling of rubber tyres (U.K.)	Calnan (1971)
		positive response on testing in 5 dairy farmers with dermatitis on the palms, exposure presumably via rubber hosing and cups in milking machines (New Zealand)	Black (1972)
		strong positive response on patch testing in student scuba divers among whom there was an epidemic of non-occupational facial dermatitis, *N-i*-propyl-*N'*-phenyl-*p*-phenylenediamine used as additive in rubber face masks	Maibach (1975)
		42 cases of occupational sensitivity; 17 tyre manufacturing, 5 tyre dealers (including 3 storage workers and 2 tyre mounters), 20 automobile transport and servicing workers (including 9 drivers, 9 mechanics, 1 petrol station attendant, 1 carriage work painter), sensitivity to other phenylenediamines also reported (see section 6.1) (France)	Hervé-Bazin et al. (1977)
		outbreak of dermatitis among 51/2000 (2.5%) post office workers using rubber finger stalls while sorting mail, 49/49 gave positive response to *N-i*-propyl-*N'*-phenyl-*p*-phenylenediamine on testing, reactions to other contituents in rubber much less frequent, presence of *N-i*-propyl-*N'*-phenyl-*p*-phenylenediamine confirmed by gas chromatography (Denmark)	Roed-Petersen et al. (1977)

Table 9/contd

Compound	Compound No.	Exposure/Response	Reference
Phenylenediamines contd		*2.2 Rubber chemicals*	
p-phenylenediamine and *N-i*-propyl-*N'*-phenyl-*p*-phenylenediamine	(33) (39)	of 9 patients with contact allergy associated with rubber products (including 4 occupationally exposed to tyres) 4 or 5 gave positive response on testing with *p*-phenylenediamine and 8 gave positive response on testing with *N-i*-propyl-*N'*-phenyl-*p*-phenylenediamine (see also naphthylamines)	Bieber and Foussereau (1968)
N-i-propyl-*N'*-phenyl-*p*-phenylenediamine *N*-phenyl-*N'*-cyclohexyl-*p*-phenylenediamine and rubber from milking machines	(39)	positive response to patch testing among 6 dairymen aged 33–64 y with hand eczema, *N-i*-propyl-*N'*-phenyl-*p*-phenylenediamine present as antioxidant in rubber tubes of milking machines (Holland)	Lintum & Nater (1974)
		2.3 Curing agents in epoxy-products	
m-phenylenediamine	(32)	positive response to patch test in male worker with allergic contact dermatitis after 5 y occupational exposure to epoxy resins and 2 y after introduction of *m*-phenylenediamine hardener, sensitivity to other aromatic amines reported (see section 6.3.4.2) but no response to epoxy resin. 4/5 co-workers also with hand dermatitis, of these 2 gave negative response to *m*-phenylenediamine and other aromatic amines (2 others not tested)	Rudzki & Krajewska (1974)
		2.4 Photographic chemicals	
4-*N*,*N*-diethyl-2-methyl-phenylenediamine monohydrochloride (CD2) and 4-(*N*-ethyl-*N*-2-methano-sulphonylaminoethyl)-2-methylphenylenediamine sesquisulphate monohydrate (CD3)		of 103/114 employees in a film laboratory, 43 exposed to chemicals, of these 21 had occupational dermatitis including 12 with contact allergy, positive response to CD2 found on patch testing in 9/23 people of whom 4 additionally positive to CD3, 2 of the CD2 sensitive individuals had lichenoid reactions as well as contact allergy (see also section 6.3.4.2)	Lidén (1984)

Table 9/contd

Compound	Compound No.	Exposure/Response	Reference
Phenylenediamines contd		*2.5 Hairdressers*	
p-phenylenediamine	(33)	positive response to patch testing among 26/84 (31%) of patients with hand eczema (U.K.)	Cronin (1980)
		positive response to patch testing among 30/66 (45%) of patients liable to occupational irritant dermatitis, sensitivity to other phenylenediamines also reported (see section 6.3.4.2) (Canada)	Lynde & Mitchell (1982)
para- hair colours		positive response to patch testing in 40% of 247 patients with eczema (Austria)	Lindemayr (1984)
Benzidines		*1 Diagnostic tests*	
benzidine	(45)	of 4600 patients tested in 5 y (1973–77), 231 (5%) were positive to benzidine, of these 38 (16%) demonstrated concomitant sensitivity to other aromatic amines; 208 (89%) of benzidine positive patients had clinically diagnosed occupational contact dermatitis, of these 153 (66%) responded uniquely to benzidine and 78 (34%) responded additionally to other allergens, occupations included workers in mechanical, construction, and textile industries, hairdressers and housewives (Barcelona, Spain)	Grimalt & Romaguera (1981)
Naphthylamines		*1 Diagnostic tests – rubber chemicals*	
N-phenyl-2-naphthylamine		positive response to testing among 6/9 patients with contact allergy associated with rubber products (including 4 occupational handling of tyres) (see phenylenediamines)	Bieber & Foussereau (1968)
Aminoazobenzenes		*1 Diagnostic tests*	
diacetylaminoazotoluene	(71)	positive skin tests among 85/4535 (1.87%) patients with contact dermatitis, use of compound as keratoplastic agent most common cause of sensitization, in 23 cases allergy considered definitely primary sensitization (i.e. not related to possible multiple hypersensitivity)	Zina & Bonu (1965)

[a] cited in IARC (1978)

p-aminobenzoic acid, procaine, benzocaine, or the sulphonamides may "cross over" to azo- and aniline-dyes (Fisher, 1973).

Persons who are allergic to one azodye are not necessarily allergic to other azodyes. Nor are persons who are allergic to *p*-phenylenediamine necessarily hypersensitive to all azodyes. Conversely, sensitivity to azodyes does not always confer sensitivity to *p*-phenylenediamine. Thus, among eight patients positive to azodyes in clothing, five reacted simultaneously to *p*-phenylenediamine (Cronin, 1968). In contrast, none of six people sensitive to Disperse Yellow 39 (dye in trousers) was positive to *p*-phenylenediamine (Sim-Davies, 1972). In a group of 125 patients with probable sensitization to industrial oils, when tested with the yellow azodye, *p*-dimethylaminoazobenzene (67), added to Polish petrol, five were positive. Among these five, three were sensitive to *p*-phenylenediamine (Rudzki et al., 1977).

Several medicaments including *p*-aminobenzoic acid, *p*-aminosalicylic acid, certain local anaesthetics (i.e. benzocaine) which are esters of *p*-aminobenzoic acid, and sulphonamides are all sensitizers which may cross-react with one another and also with *p*-phenylenediamine and azo- and aniline-dyes. Benzocaine, although a fairly weak sensitizer, is the most potent of the typical sensitizing anaesthetics. Besides allergic contact dermatitis of the delayed type, it can also cause contact urticaria (Ryan et al., 1980). Apparent cross-reactions can occur between the antibacterial sulphonamides and chemically related compounds that are used as diuretics, oral treatments for hypoglycaemia or sweetening agents. Topical application of a sulphonamide may sensitize the patient so that subsequent systemic administration of a sulphonamide may produce contact-type dermatitis medicamentosa. The salts of *p*-aminobenzoic acid used in sunscreens occasionally sensitize but the acid itself is not so potent a sensitizer. *p*-Aminobenzoic acid and sulphonamides are also photosensitizers.

Several aromatic amines used as rubber additives are allergenic. In contrast to plastic which when completely cured is totally innocuous to the skin, rubber may cause allergic contact dermatitis even after complete curing probably due to antioxidants on the rubber surface. Small quantities of the antioxidants may be released over many years (Adams 1983). A descriptive illustration of how sensitizing chemicals "bloom" on to the surface of rubber has been provided by Fregert (1973).

As additives are present also in finished products, both consumers and manufacturers are at risk of being sensitized. Industrial rubber chemicals often include compounds in the *p*-phenylenediamine and naphthylamine groups, but this is not generally the case for domestic rubbers. A number of domestic rubber hypersensitivity patients are seen at the dermatological departments in various hospitals. In contrast, comparatively few patients who have been sensitized by industrial rubber chemicals are seen. During the decade 1967-1976 the average was 11 patients a year at St John's Hospital, London. As the exposure was mainly occupational, more men than women were sensitized (Cronin, 1980).

Epoxy hardeners also are frequent allergens. According to Foussereau et al. (1982), they are responsible for 15% to 30% of all cases of epoxy allergy. Cyclic and aromatic amines are particularly frequent sensitizers. Fully cured epoxy products are harmless.

6.2 Human cancers associated with possible exposure to aromatic amines

Several epidemiological studies have reported populations with an elevated incidence of bladder cancer possibly associated with exposure to aromatic amines. It has also

been proposed that some situations leading to possible exposure to aromatic amines are associated with an increased risk of cancers at other sites. Reports in which the disease has been strongly associated with exposure to particular chemicals, as in the dye industry, or some studies in the rubber industry are discussed in Sections 6.3–6.7.

Case & Hosker (1954) observed an excess risk of death from bladder cancer in the rubber industry between 1936 and 1951 (26 found, 15.9 expected), but could demonstrate no such risk for 1921-1935. The authors proposed that the excess bladder cancer risk found in later years may have been due to the introduction around 1927-1928 of an antioxidant contaminated with naphthylamine isomers. Fox & Collier (1976), however, observed that although there was an excess risk of death from cancer in the rubber and cable-making industry, including an elevated risk of death from bladder cancer, the latter was not associated only with those employed prior to 1950 in plants where rubber additives manufactured from and contaminated with naphthylamine isomers were in use. An elevated risk of death from bladder cancer was also found for people employed in such plants only after the discontinuation of the use of the contaminated additives in 1949, or employed where known carcinogens had never been used. An excess risk of death from lung or stomach cancer was also observed, in agreement with several other reports (see Fox & Collier, 1976; Nutt, 1983).

Other occupations for which an elevated risk of bladder cancer has been reported include the gas, coke, chemical, textiles and clothing industries (Anthony, 1974) with a relative risk as high as 8.1 for dye-workers. Cartwright (1983) also reports a low excess risk of bladder cancer for clerical workers, cooks, machinists and woodworkers, and comments that although the risk of bladder cancer in arylamine-exposed dye-workers is still high, it is largely historical in that the risk ratio decreases as the time of first exposure becomes more recent. The apparent risk in recent years, however, may be understated due to the latency of the disease.

Recent studies have implicated a broad spectrum of aromatic amines in both acute and chronic hair dye toxicity (IARC, 1978; Marzulli et al., 1978). Several epidemiological studies have indicated that there may be an increase in certain cancers among users of hair dyes and those exposed to hair preparations in hair-service occupations (i.e. hairdressers, beauticians, barbers, etc.). The results of 4 of 5 case-control studies of bladder cancer were consistent with an increased risk for hairdressers and barbers (IARC, 1978). Garfinkel et al. (1977) and Menck et al. (1977) reported an elevated risk of lung cancer amongst beauticians. Additional data from population-based death and cancer registries have also indicated an increased occupational risk among barbers and beauticians for cancer at different sites (IARC, 1978). Insufficient epidemiological data are currently available to assess the cancer risk due to the personal use of hair dyes (Van Duuren, 1980). The contribution of aromatic amines in hair dye formulations to increased cancer risk is complicated by the confounding effects due to smoking and exposure to other toxic substances, for example, to aerosal propellants (such as vinyl chloride and fluorocarbons).

Cigarette smoking is also associated with an increased risk of bladder cancer (Cole et al., 1971; Armstrong et al., 1976; Wynder & Goldsmith, 1977; Moolgavkar & Stevens, 1981) and both 1- (57) and 2-naphthylamine (58) have been found in cigarette smoke, although it is not clear how significant the levels found may be with respect to cigarette smoking toxicity (Hoffman et al., 1969). The relative risk for both sexes has been reported to be as high as 3, with an estimate for 1966–1970 that 27% of female bladder cancer deaths and 85% of male bladder cancer deaths in England

and Wales were directly attributable to smoking. Smoking has also been associated with an increased risk of carcinoma of the pancreas, and an observed increase in mortality in both diseases in England and Wales over recent years is attributable almost entirely to increases among smokers. Were it not for smoking, bladder cancer incidence would have decreased greatly in these countries between 1941 and 1970.

Several estimates of the degree of occupational involvement in human bladder cancer incidence have been made, ranging from 10–50%. It has been proposed, for example, that in the metropolitan Boston area 50–60% of human bladder cancer may be directly attributable to occupational or cultural environments (for example, smoking) where there is potential exposure to aromatic amines (Lower, 1982).

6.3 Anilines and phenylenediamines

6.3.1 Pharmacokinetics and metabolism

6.3.1.1 Animals. Aniline (1) is rapidly absorbed by experimental animals following exposure via oral administration, skin application or inhalation (IARC, 1982a). Studies on the percutaneous absorption of possible hair-dye components have demonstrated some absorption of *m*- (32) and *p*-phenylenediamine (33) and 2,5-diaminotoluene (35) in dogs (IARC, 1978), and 2,4-diaminotoluene (34) in monkeys (Marzulli et al., 1978).

Following administration of radiolabelled aniline to rats, the radioactivity is distributed throughout the body with the highest levels being found in the blood, liver, kidney, bladder and gut (IARC, 1982a).

Table 10 summarizes the metabolites identified in the urine of experimental animals following administration of some anilines and phenylenediamines. When the free amine has been isolated in urine, generally only small amounts have been found although for 2,4,5- (10) and 2,4,6-trimethylaniline (11) the parent amine was the major urinary product (30% and 15% dose respectively) following oral administration to rats. Ring hydroxylated products are not always found but when they are, they are frequently the major isolated urinary metabolites. Oxidation of the ring-methyl to a benzyl alcohol or benzoic acid is observed for ring-methylated anilines; in addition to those described in Table 10, trace amounts of the benzoic acid and quinone derivatives have been isolated in rat urine following administration of 2,4,5- and 2,4,6-trimethylaniline.Demethylation has been observed for 2,4-diaminoanisole (36) *in vivo*, and *o*- (12) and *p*-anisidine (13) are dealkylated by rat liver microsomes *in vitro*. Metabolites may be excreted as *N*-conjugates. *N*-acetylation and glycine or glucuronic acid conjugation of the carboxy group are major metabolic pathways for *p*-aminobenzoic acid (17). In rats the extent of acetylation is inversely related to dose, and there is also an inverse relationship between acetylation and glycine conjugation. Despite the fact that dogs are generally regarded to be poor acetylators, *N,N*-diacetyl-*p*-phenylenediamine has been reported in dog urine following administration of *p*-phenylenediamine. Although phenylhydroxylamine has not been identified in the urine of any experimental animals administered aniline, both phenylhydroxylamine and nitrosobenzene have been detected in the blood of treated cats and dogs. Phenylhydroxylamine appears to be the major cause of methaemoglobinaemia following aniline administration. *N*-oxidized metabolites have rarely been reported in the urine of animals administered anilines or phenylenediamines, small amounts of azoxytoluene and nitrosotoluene have been

Table 10 Summary of identified urinary metabolites of some monocyclic aromatic amines[a, b]

Pathway	Metabolites		
	aniline	*o*-toluidine	2,4-dimethylaniline
unmetabolized	aniline[c]	*o*-toluidine	2,4-dimethylaniline
N-sulphonation	phenylsulphamic acid[c]		2,4-dimethylphenylsulphamate
N-glucuronidation	aniline-*N*-glucuronide[c]		
ring hydroxylation/ conjugation	*o*-aminophenol[d]	2-amino-*m*-cresol (sulphate conjugate)	
	m-aminophenol[e]	4-amino-*m*-cresol (also as sulphate and glucuronide conjugates) (major)	
	p-aminophenol[d]		
	o-aminophenylmercapturic acid		
	p-aminophenylmercapturic acid		
	p-acetylaminophenol[f]	*N*-acetyl-4-amino-*m*-cresol (also as sulphate and glucuronide conjugates)	
	p-acetylaminophenylmercapturic acid[g]		
N-acetylation	acetanilide[f]		2,4-dimethylacetanilide
		N-acetyltoluidine	
oxidation of methyl		*N*-acetyl-*o*-aminobenzylalcohol (also glucuronide conjugate)	3-methyl-4-acetamidobenzoic acid (major)
		N-acetylanthranilic acid	
		anthranilic acid	3-methyl-4-aminobenzoic acid (also as glycine conjugate)

Table 10/contd

Pathway	Metabolites		
	2,5-dimethylaniline	2,4-diaminotoluene	2,4-diaminoanisole
unmetabolized *N*-sulphonation *N*-glucuronidation	4-hydroxy-2,5-dimethylaniline	2,4-diamino-5-hydroxytoluene[c] (major)	5-hydroxy-2,4-diacetylaminoanisole
ring hydroxylation/ conjugation			
N-acetylation		4-acetylamino-2-aminotoluene 2,4-diacetylaminotoluene	4-acetylamino-2-aminoanisole 2-methoxy-5-glycolamidoacetanilide 2,4-diacetylaminophenol
oxidation of methyl	4-methyl-2-aminobenzoic acid 4-methyl-3-aminobenzoic acid	2,4-diacetylaminobenzoic acid	

[a] From IARC (1978, 1982a)

[b] isolated in rat urine following dosing with parent amine unless otherwise specified

[c] species not specified

[d] isolated in rat, rabbit, mouse, guinea pig, gerbil, hamster,

[e] isolated in rabbit and dog urine

[f] isolated in rabbit urine

[g] isolated in rat and rabbit urine

isolated from the urine of rats following administration of *o*-toluidine (2) (0.2% and 0.1% dose respectively) (IARC, 1978, 1982a).

Following administration of ^{14}C-labelled *o*-toluidine, 2,4-diaminotoluene and 2,4-diaminoanisole to rats (via s.c., i.p. and i.p. routes respectively) (IARC, 1978, 1982a) and 2,4-diaminotoluene to guinea pigs (i.p. route) (Marzulli et al., 1978) the majority of an administered dose was eliminated in the urine (80% in 2 or 5 days), with smaller amounts in the faeces.

6.3.1.2 Humans In humans, aniline (1) vapour may be absorbed via the skin and the respiratory tract, as is demonstrated by the urinary excretion of *p*-aminophenol conjugates. *o*-Toluidine (2) is also absorbed via the skin and respiratory tract (IARC, 1982a). Urinary excretion of the parent amine or metabolites has demonstrated percutaneous absorption for *p*-phenylenediamine (33), 2,4- (34) and 2,5-diaminotoluene (35) and 2,4-diaminoanisole (36). 2,4-Diaminoanisole appears to penetrate the skin less readily than the other diamines tested, and peak penetration was reported to occur 24-48 h after application (Marzulli et al., 1978).

N-Acetylation has been demonstrated following s.c. and dermal administration of 2,5-diaminotoluene (IARC, 1978; Marzulli et al., 1978) and oral administration of *p*-aminobenzoic acid (17) (IARC, 1978). The fraction of acetylated metabolites of *p*-aminobenzoic acid decreased with increasing doses of amine. Glycine conjugation of *p*-aminobenzoic acid to form hippuric acid and the related *N*-acetylated conjugate has also been observed. Concurrent administration of sodium benzoate completely eliminated the excretion of the glycine conjugates. Glycine conjugation is low in neonates (IARC, 1978).

Methaemoglobin production caused by aniline in humans is probably as a result of *N*-hydroxylation (IARC, 1982a).

Following skin application of ^{14}C-labelled *p*-phenylenediamine, 2,4-diaminotoluene and 2,4-diaminoanisole, urinary excretion of the radiolabel was observed, although smaller amounts of 2,4-diaminotoluene were excreted than the other two phenylenediamines (Marzulli et al., 1978). Urinary excretion of approximately half of an s.c.dose of 2,5-diaminotoluene (largely as the acetylated metabolite) has also been observed (IARC, 1978).

6.3.2 Short-term tests

Table 11 summarizes data on mutagenicity and other short-term assays for genotoxicity of anilines. Three major end points are considered, DNA damage, mutagenicity and chromosomal anomalies. DNA damage includes covalent binding to DNA, induction of DNA breakage or repair, induction of prophage in bacteria and responses in DNA repair-proficient or repair-deficient bacteria. Mutagenicity includes mutations in cultured cells or organisms such as forward or reverse point mutations, recombination, gene conversion and specific locus mutations. Chromosomal anomalies include aberrations, breaks and gaps, etc., sister chromatid exchange, micronuclei and aneuploidy. Each cross in the table represents a result in 1 or more short-term genetic toxicity tests in 1 or more biological systems.

Table 11 Short-term tests: anilines and phenylenediamines

Compound	Compound No.	Positive results				Negative results			
		DNA damage	Mutagenicity	Chromosome anomalies	Other	DNA damage	Mutagenicity	Chromosome anomalies	Other
Anilines									
aniline[a,b]	(1)		X	X		X	X	X	$X_{CT\,SA}$
o-toluidine[a]	(2)	X	X	X	X_{CT}		X	X	
m-toluidine[c]	(3)						X		
p-toluidine[c]	(4)					X	X		
2,4-xylidine[c]	(5)		X			X			
2,5-xylidine[c]	(6)		X			X			
2,4,5-trimethylaniline[a]	(10)		X			X			
2,4,6-trimethylaniline[a]	(11)	X					X		
o-anisidine[a]	(12)		X						
p-anisidine[a]	(13)						X		X_{CT}
m-cresidine[a]	(14)						X		
p-cresidine[a]	(15)		X						
anthranilic acid[d]	(16)			X					
m-aminobenzoic acid[b]							X		
p-aminobenzoic acid[d]	(17)						X		
phenacetin[e]	(19)		X	X			X	X	
m-nitroaniline[b]	(22)		X						
p-nitroaniline[b]	(23)		X						
2,4-dinitroaniline[b]			X						
4-amino-2-nitrophenol[d]	(24)		X						
2-amino-4-nitrophenol[b]			X						
5-nitro-*o*-anisidine[a]	(25)		X						
2,4,6-trichloroaniline[c]	(29)						X		
p-chloro-*o*-toluidine[c,d]	(30)	X	X						
o-aminophenol[b]							X		
m-aminophenol[b]							X	X	
p-aminophenol[b]			X	X	X_{SA}		X		
2-methyl-4-aminophenol[b]							X		

Table 11/contd

Compound	Compound No.	Positive results				Negative results			
		DNA damage	Mutagenicity	Chromosome anomalies	Other	DNA damage	Mutagenicity	Chromosome anomalies	Other
Phenylenediamines									
o-phenylenediamine[f,g]	(31)		X	X					X_{DL}
m-phenylenediamine[b,d]	(32)		X					X	X_{DL}
p-phenylenediamine[b,d,h]	(33)		X					X	
2,4-diaminotoluene[i]	(34)	X	X	X	X_{CT}			X	X_{DL}
2,5-diaminotoluene[b,d]	(35)		X					X	X_{DL}
2,6-diaminotoluene[i]		X	X				X		
2,4-diaminoanisole[d]	(36)		X				X		X_{DL}
1,2-diamino-4-nitrobenzene[d,i]	(40)	X	X	X	X_{CT}			X	X_{DL}
1,2-diamino-2-nitrobenzene[d,j]	(41)	X	X	X	X_{CT}				X_{DL}
4-chloro-*o*-phenylenediamine[a]	(42)	X							
2,6-dichloro-*p*-phenylene--diamine[i]			X						
1,2,4-triaminobenzene[b]			X						X_{SA}

From:

[a] IARC (1982a)

[b] Combes & Haveland-Smith (1982)

[c] Zimmer et al. (1980)

[d] IARC (1978)

[e] IARC (1982c)

[f] Wild et al. (1980)

[g] Ames et al. (1975)

[h] Blijlevan (1977)

[i] IARC (1986)

[j] Martin et al. (1978)

Key:
CT cell transformation
SA sperm abnormality
DL dominant lethal

6.3.3 Carcinogenicity in experimental animals

IARC has determined that there is sufficient evidence for carcinogenicity in experimental animals for *o*-toluidine (2), *o*-anisidine (12), *p*-cresidine (15), phenacetin (19), *p*-chloro-*o*-toluidine (30) 2,4-diaminotoluene (34), 2,4-diaminoanisole (36) and 4-chloro-*o*-phenylenediamine (42), and limited evidence for aniline (1), 2,4, 5-trimethylaniline (10), analgesic mixtures containing phenacetin (19), 5-nitro-*o*-anisidine (25) and 2,6-dichloro-*p*-phenylenediamine (IARC, 1978, 1982a,c, 1983, 1986).

Other anilines and phenylenediamines have been tested for carcinogenicity in the NCI Bioassay Program but have not been evaluated by IARC. These include the anilines: *m*-toluidine (3), *p*-toluidine (4), 3-chloro-*p*-toluidine, 5-chloro-*o*-toluidine, 5-nitro-*o*-toluidine, *p*-chloroaniline (27), 2,4,6-trichloroaniline (29) 3,4-dimethoxyaniline, 4-nitroanthranilic acid and 3-nitro-4-ethoxy-*N*-acetylaniline and the phenylenediamines: *o*-phenylenediamine (31), 2-chloro-*p*-phenylenediamine (44), 2,6-diaminotoluene, tetrafluoro-*m*-phenylenediamine, *N*-phenyl-*p*-phenylenediamine (38) and 3-amino-4-ethoxy-*N*-acetylaniline (Weisburger et al., 1978; Milman & Peterson, 1984; Lavenhar and Maczka, 1985).

The terms "sufficient" and "limited" evidence refer to the IARC definitions (IARC, 1982c). Sufficient evidence indicates an increased incidence of malignant tumours in multiple species or strains or in multiple experiments, preferably with different routes of administration or an unusual degree of tumour incidence, site or type of tumour or age at onset of disease. Limited evidence indicates positive results in a single species, strain or experiment, or experiments limited by inadequate dosing, duration, follow-up, survival, numbers of animals or reporting, or the occurrence of tumours which often occur spontaneously. Compounds that have been evaluated by IARC, where "inadequate" evidence existed to demonstrate either the presence or absence of a carcinogenic effect, have not been referred to unless results are considered important in the light of unclear evidence of carcinogenicity in humans. (This applies to all sections on 'carcinogenicity in experimental animals').

6.3.4 Effects in humans

6.3.4.1 Systemic An often characteristic symptom of poisoning by aromatic amines is methaemoglobinaemia. Occupational aniline (1) poisoning was once not uncommon; acute intoxication could cause methaemoglobinaemia resulting in cyanosis and possibly death from asphyxiation (ACGIH, 1980). The presence of Heinz bodies, (small refractile granules in the erythrocytes), has also been reported. Symptoms of toxicity may include headache, dizziness and nausea (Jenkins et al., 1972). Acute poisoning by *o*-toluidine (2) may also cause methaemoglobinaemia (IARC, 1982a). Single oral doses of 5 or 15 mg aniline had no effect in 20 human volunteers. Increasing the dose to 25−65 mg/person caused a dose-dependent increase in methaemoglobin formation. The higher doses (45−65 mg/person) also caused a slight increase in serum bilirubin in two males. No Heinz bodies were detected at any dose (Jenkins et al., 1972). The hazard associated with industrial exposures to aromatic amines, however, is related to their physical properties. For example, whereas aniline is fat-soluble and therefore readily penetrates the skin, clothing and shoes, so that

a small area of contamination on clothing or gloves may cause poisoning if left in contact with the skin for several hours, occupational methaemoglobinaemia has not been reported to be caused by 2,4-diaminotoluene (34) or *m*-phenylenediamine (32) which are not fat-soluble and have low vapour pressures (Hamblin, 1963). Exposure to aniline or *o*-toluidine vapour levels of 7−53 ppm for several hours may cause slight symptoms. More than 1 h exposure to aniline levels of 100−160 ppm may cause serious disturbances. At least 1 death from liver damage has been reported following aniline poisoning (ACGIH, 1980).

Although the occurrence of chronic aniline poisoning is disputed, nervous system disorders have been reported following long-term exposure both to aniline and *o*-toluidine, although in the latter case reported symptoms have been less severe. A reduction in red blood cells, haematuria, anuria and liver damage also have been associated with long-term *o*-toluidine exposure (ACGIH, 1980).

Several outbreaks of chloracne associated with the handling of 3,4-dichloroaniline (28) or its derivatives and related herbicides have been reported (Sundström, 1980). The probable causative chloracnegenic agents were 3,3′,4,4′-tetrachloroazobenzene and 3,3′,4,4′-tetrachloroazoxybenzene which may be formed either during the synthesis of 3,4-dichloroaniline or its conversion to herbicides. The acnegenic chlorinated azo and azoxybenzenes are structurally related to the well documented chloracnegens, chlorinated dibenzo-*p*-dioxins and dibenzofurans.

Concern has been expressed over the possible toxicity of aromatic amines used in hair dye preparations. Aplastic anaemia has been reported following the use of hair dyes containing 2,5-diaminoanisole (37). Symptoms of poisoning possibly associated with *p*-phenylenediamine (33), following the use of hair dye preparations containing the amine, have been reported to include neurological disturbances such as vertigo and diplopia, asthenia, gastro-intestinal disorders, splenic enlargement and hepatic damage. Fatal poisoning has been reported (IARC, 1978).

6.3.4.2 Dermatitis *p*-Phenylenediamine (33) is generally classified as a common cause of positive responses in patch test clinics (Magnusson et al., 1968; Calnan et al., 1970; Baer et al., 1973; Rudner et al;. 1973). It also scores high in predictive studies in animals. Results from some prevalence studies and diagnostic tests are summarized in Table 9.

Sensitization by *p*-phenylenediamine has been considered so great a hazard that its use in hair dyes has been banned in several European countries. Not counting beauticians and hairdressers who contact *p*-phenylenediamine as an occupational problem and where it can be disabling (James & Calnan, 1959; Fregert, 1975; Cronin, 1980), comparatively few cases of sensitization appear per year. This might be explained by a comparatively short time of exposure of *p*-phenylenediamine to the previously intact skin in people who dye their own hair. *Para*-dyes once fully polymerized on the hair are said to be inert and harmless (Reiss & Fisher, 1974). Thus dyed hair is usually non-allergic and does not cause dermatitis in those sensitized. This observation is of particular importance to hairdressers who frequently handle dyed hair. However, this is only true if the hair has been correctly dyed and all the amine is oxidized (Hindson, 1975). Immediate hypersensitivity to *p*-phenylenediamine has also been reported (Cronin, 1980).

A number of derivatives of *p*-phenylenediamine have also been used as hair dyes. *o*-Phenylenediamine (31) and *p*-phenylenediamine, 2,5-diaminotoluene (35), 2,4- and 2,5-diaminoanisole, *N*-phenyl-*p*-phenylenediamine (38), 1,4-diamino-2-nitro-

benzene (41), *o*-aminophenol, and *p*-diaminophenol all have sensitizing capacity (Ludwig, 1982) and, for example, 10 of the 30 hairdressers sensitized by *p*-phenylenediamine reported by Lynde & Mitchell (1982) (Table 9) were also sensitive to 2,5-diaminotoluene (5 patients), *N*-phenyl-*p*-phenylenediamine (3 patients) or 1,4-diamino-2-nitrobenzene (2 patients). The most widely used hair dye apart from *p*-phenylenediamine is 2,5-diaminotoluene which has never been banned as it has always been considered a weaker allergen than *p*-phenylenediamine (Cronin, 1980). However, the evidence for this is inconclusive. A predictive test by Epstein & Taylor (1979) in human volunteers, comparing sensitization to *p*-phenylenediamine (Table 9) and 2,5-diaminotoluene, resulted in a complete lack of sensitization to the latter compound. In contrast, 2,5-diaminotoluene was reported by Hjorth to be a sensitizer in a group of almost 2,000 patients with a potency of about half that of *p*-phenylenediamine (cited in Epstein & Taylor, 1979).

Many of the derivatives of aniline (1) and *p*-phenylenediamine used as additives (e.g. antioxidants) in rubber manufacturing processes are allergenic although rubber itself is not. Some cases of dermatitis from rubber products, notably those due to *N*-*i*-propyl-*N'*-phenyl-*p*-phenylenediamine (39), are outlined in Table 9. *N*-*i*-propyl-*N'*-phenyl-*p*-phenylenediamine is both one of the most frequently used *p*-phenylenediamine derivatives in the rubber industry and generally found to be the most potent sensitizer in animal experiments among the rubber chemicals related to *p*-phenylenediamine (Schonning & Hjorth 1968; Cronin, 1980; Korossy et al., 1981). *N*-*i*-Propyl-*N'*-phenyl-*p*-phenylenediamine sensitivity has been found in dairy farmers (Table 9). It is one of the chemicals allowed in dairy rubber by German regulations which are adhered to also by most manufacturers of dairy rubber for the European Common Market (Lintum & Nater, 1974). *N*-Dimethyl-1,3-butyl-*N'*-phenyl-*p*-phenylenediamine which is an antioxidant similar to *N*-*i*-propyl-*N'*-phenyl-*p*-phenylenediamine has previously been considered as a non-sensitizer; however, investigations by Hervé-Bazin (1977) in experimental animals revealed that the two compounds have similar allergenicity.

m-Phenylenediamine (32), a well-known curing agent for epoxy resins, is a strong irritant and allergic sensitizer (Table 9). It also stains the skin and nails yellow. Rudzki & Krajewska (1974) reported sensitivity to aniline, *p*-phenylenediamine, toluidine and benzidine (45) as well as to *m*-phenylenediamine following exposure to epoxy resin (Table 9).

It has long been known that chemicals used in the development of colour and black and white film may cause skin diseases. Many of the colour developing agents that are derivatives of *p*-phenylenediamine are irritant and also cause contact allergy. Sensitization from these chemicals has been reported with CD2 (4-*N*,*N*-diethyl-2-methylphenylenediamine monohydrochloride) (Mandel, 1960; Knudsen, 1964; Fry, 1965; Miranda et al., 1978), and occasionally from CD3 (4-(*N*-ethyl-*N*-2-methanesulphonylaminoethyl)-2-methylphenylenediamine sesquisulphate monohydrate) (Fry, 1965), Agfa TSS (4-amino-*N*-diethylaniline sulphate) (Knudsen, 1964, and MI 210 (*N*-ethyl-*N*-(5-hydroxy-amyl)-*p*-phenylenediamine hydrogen sulphate) (Fry, 1965).

In the early days, before the use of preventive measures, sensitization from colour developers was frequent. An incidence of 25% in one plant was quoted by Buckley (1958), and Knudsen (1964) stated that 50% of those exposed were affected. The findings of an investigation of occupational dermatoses following exposure to CD2 and CD3 at a film laboratory are summarized in Table 9. CD2 is apparently a more potent sensitizer than CD3. Whether cross-reaction between CD2 and CD3 occurs

is difficult to say in view of simultaneous exposure to both chemicals. Of the nine individuals sensitive to CD2, two also exhibited sensitivity to *p*-phenylenediamine (Lidén, 1984). Since the 1950s, it has been known that certain colour developers also may give rise to lichenoid reactions or lichen planus. In earlier investigations, lichenoid reactions to CD2 and CD3 were more frequent than eczema (Buckley, 1958; Canizares, 1959; Mandel, 1960; De Graciansky & Boulle, 1966; Czerwinska-Dihm, 1977; Kersey & Stevenson, 1980). This was not the case in Lidén's study. Why exposure to CD2 gives rise to eczema in some people and to lichenoid reaction in others is unclear. CD2 may have a greater tendency to provoke lichenoid reaction than other colour developing agents.

6.3.4.3 Carcinogenicity Historically, many cases of bladder cancer were reported in people involved in the manufacture of aniline (1). The possibility of simultaneous exposure to other carcinogenic compounds, however, generally was not addressed. In the early 20th century, the aniline in use contained as impurities many compounds now recognized to be human bladder carcinogens.

There have been several reports in the literature concerning populations occupationally exposed to aniline; some are summarized in Table 12. Sufficient evidence to indicate an increased risk of bladder cancer has not been demonstrated, and some studies report no cases of bladder tumours in exposed populations. Many of the reports apparently do not take adequate account of concomitant exposures to other potential carcinogens, nor do some indicate the size of the exposed population (IARC, 1982a).

Several reports on carcinogenic risk to humans following occupational exposure to *o*-toluidine (2) have been summarized by IARC (1982a). From a knowledge of the aniline-dye production process, it is likely that the reports by Case et al. (1954) and Case & Pearson (1954) on bladder cancer incidence in aniline workers included individuals who were also exposed to toluidines. Similarly, it is likely that some workers exposed to other aromatic amines, including 1-naphthylamine (57) and benzidine (45), who did develop bladder cancer were exposed additionally to *o*-toluidine. Most reports on populations exposed to toluidines have surveyed groups with mixed exposures to other aromatic amines. Khlebnikova et al. (see Table 12) and Lipkin (1972) have reported bladder cancers in groups exposed to toluidines, but in the former case it is not clear whether or not there was concomitant exposure to other potentially carcinogenic agents, and in the latter case the size of the exposed population was not given. In contrast, in a study of the occurrence of bladder tumours in workers producing dyestuff intermediates, no bladder cancers were diagnosed among 35 men exposed to *o*-toluidine during the preparation of *p*-chloro-*o*-toluidine (30). They may additionally have been exposed to other aromatic amines (IARC, 1982a). There is some evidence for a longer latent period and age of onset of bladder cancer following combined exposure to aniline/toluidine, relative to exposure to 2-naphthylamine (58) or benzidine (IARC, 1982a).

o-Toluidine is used in conjunction with 4,4′-methylenebis(2-methylaniline) (55) in the manufacture of the dye magenta ABN (New Fuchsin), a process which together with the manufacture of safranine-T has been associated with an elevated risk of death from bladder cancer (Rubino et al., 1979). As part of a survey of employees in an Italian dyestuff factory, five deaths from bladder cancer among magenta ABN and safranine-T exposed employees were recorded (0.08 expected). Three deaths

Table 12 Major systemic human health effects following known exposure to aromatic amines

Compound	Compound No.	Exposure	Disease/Number affected	References
Anilines				
aniline (possible concomitant exposure to other aromatic amines)	(1)	manufacture of aniline	1934-1947, out of 99 cases bladder tumour diagnosed in ~4000 employees from two U.K. chemical factories, 3 were cases of papilloma in men exposed only to aniline (total population exposed to aniline alone not cited)	Goldblatt (1949)[a]
aniline	(1)	occupational exposure during manufacture and use	3 cases of bladder cancer associated with aniline in British chemical industry 1910-1952, all in a group known to be exposed due to employment in firms manufacturing aniline. 1 death for which a death certificate mentions bladder cancer (0.52 such certificates expected)	Case & Pearson (1954)
o- and/or *p*-toluidine	(2) (3)	production	1960s, U.S.S.R., 2 cases of bladder cancer diagnosed following cystoscopic examination of 75/81 current toluidine workers (19 women, 62 men, aged 22-55, 9 exposed < 1 y, 31 exposed 1-5 y, 41 exposed > 5 y) 1 diagnosed following 20 m exposure to *p*-toluidine, the other following 23 y exposure to both isomers 6/16 former employees (12-17 y exposure) had bladder tumours	Khlebnikova et al. (1970)[a]

Table 12/contd

Compound	Compound No.	Exposure	Disease/Number affected	References
Anilines				
m-phenylenediamine	(32)	production	employees aged 30-50 y, 5-10 y exposure, ‡3.4% had dysuria, 8% positive in scratch test, abnormalities found on cystoscopy	Orlov (1974)[b]
Benzidines				
benzidine	(45)	occupational exposure in dye factories	total incidence of bladder carcinoma = 4.6%, incidence of bladder papillomas = 8.1%, duration of employment before appearance of tumours ranged from 5-26 years	Di Maio (1937)[c] Barsotti & Vigliani (1949)[c]
benzidine	(45)	dyestuffs manufacturing plant	incidence of bladder tumours = 10.6% mean duration of exposure = 15.9 years	Scott (1952)[c]
benzidine	(45)	occupational exposure during manufacture and use, may also have been exposed to aniline	38 cases of bladder cancer associated with benzidine diagnosed in British chemical industry 1900-1952, of these 34 were in a group of 496 known to be exposed due to employment in firms manufacturing benzidine, 13 of the 34 were dead, of these 10 death certificates mentioned bladder cancer (0.54 such certificates expected) expected final bladder cancer incidence = 13%, mean latent period from time of first exposure to tumour development = 16 years	Case et al. (1954)

Table 12/contd

Compound	Compound No.	Exposure	Disease/Number affected	References
benzidine	(45)	coal-tar dye production (workers exposed to benzidine alone)	incidence of bladder malignancies = 22.3% mean induction period = 18.7 years	Goldwater et al. (1965)
benzidine	(45)	occupational exposure during manufacture	attack rate for kidney and bladder cancers = 237/100 000 person years, among white males 25-64 employed prior to 1940	Mancuso & El-Attar (1967)
benzidine	(45)	benzidine manufacture	total incidence of bladder tumours = 52% mean duration of exposure in workers who developed tumours = 13.6 years mean induction period = 16.6 years	Zavon et al. (1973)[c]
3,3′-dichlorobenzidine	(50)	occupational	3 retrospective epidemiological studies of workers did not reveal an increased incidence of bladder tumours. A variety of other tumours (including 6 lipomate) were reported	Gerarde & Gerarde (1974) Gadian (1975)[c] MacIntyre (1975)[c]
benzidine-based dyes (multiple exposure)		silk-dying industry	at least 10/17 patients with bladder cancer were kimono painters	Yoshido et al. (1971)
benzidine-based dyes (Direct Black 38 and Direct Blue 2)		drying and grinding procedures in direct azo dye manufacture	retrospective epidemiological study revealed 5 cases of bladder cancer exposed period range 3-24 years	Genin (1977)

Table 12/contd

Compound	Compound No.	Exposure	Disease/Number affected	References
4-aminobiphenyl	(72)	occupational	total incidence of bladder tumours = 11.1% duration of exposure ranged from 1.5-19 years	Melick et al. (1955)[d]
4-aminobiphenyl	(72)	occupational	in a 17-year follow-up study incidence of bladder tumours = 17% (exact duration of exposure not established)	Melick et al. (1971)[d]
4,4'-Diaminodiphenylmethanes				
4,4'-methylenedianiline	(53)	used as epoxy resin hardener, exposure when hardener powdered or when manually mixed into resin on heated rollers	over 6-year period 13 diagnosed as having jaundice out of approximately 100 similarly exposed, additional 4 with symptoms compatible with jaundice	McGill & Motto (1974)
4,4'-methylenedianiline	(53)	mixed with liquid epoxide and sprayed or spread onto concrete walls as surface coat	6/300 developed jaundice within 2 days to 2 weeks of exposure	Williams et al. (1974)
4,4'-methylenebis-(2-chloroaniline)	(54)	demonstrated absorbtion (as assessed by urinary excretion of parent compound and metabolites) following exposure during manufacturing process	no cytological evidence of bladder cancer risk in 31 people, average age 50, exposed 6 months—16 years, still exposed at time of survey No cases of bladder cancer reported in 178 people exposed at some time but not for at least 10 years out of a total workforce of 6314	Linch et al. (1971)

Table 12/contd

Compound	Compound No.	Exposure	Disease/Number affected	References
Naphthylamines				
1-naphthylamine (commercial)	(57)	occupational exposure during manufacture and use (commercial product containing 4-10% 2-naphthylamine) may also have been exposed to aniline	bladder tumours induced only following more than 5 years exposure. 28 cases of bladder cancer associated with commercial 1-naphthylamine diagnosed in British chemical industry 1900-1952; of these 19 were in a group of 609 known to be exposed due to employment in firms manufacturing 1-naphthylamine, 6 of the 19 were dead, death certificates of these mentioned bladder cancer (0.66 such certificates expected) expected final bladder cancer incidence = 11% mean latent period from time of first exposure to tumour development = 22 years	Case et al. (1954)
2-naphthylamine	(58)	occupational exposure during manufacture and use, may also have been exposed to aniline	59 cases of bladder cancer associated with 2-naphthylamine diagnosed in British chemical industry 1900-1952; of these 55 were in a group of 218 known to be exposed due to employment in firms manufacturing 2-naphthylamine, 27 of the 55 were dead, of which 26 death certificates mentioned bladder cancer (0.3 such certificates expected) expected final bladder cancer incidence = 43% all of those employed distilling 2-naphthylamine died from bladder cancer mean latent period from time of first exposure to tumour development = 16 years	Case et al. (1954) Case (1956)

Table 12/contd

Compound	Compound No.	Exposure	Disease/Number affected	References
Naphthylamines contd				
2-naphthylamine	(58)	coal-tar dye plant	12/48 exposed developed bladder cancer mean survival following diagnosis = 8 years	Goldwater et al. (1965)
2-naphthylamine	(58)	occupational exposure during manufacture	attack rate for bladder or kidney cancer = 952/100 000 person-years, among white males 25-64 employed prior to 1940	Mancuso & El-Attar (1967)
2-naphthylamine (in Nonox S)	(58)	rubber tyre factory	23 bladder tumours (10.3 expected) out of 2081 exposed during 1946-1949, follow-up to 1970	Veys (1973)
Aldol-α-naphthylamine (Algerite) contaminated with high levels of free naphthylamines and/or condensation product of paraldehyde and 1- and 2-naphthylamine (Nonox S) contaminated with free naphthylamine		Tyre factory-material used as rubber antioxidant	1948-1967 follow-up of 20 000 employed at factory since 1930, 1400 were exposed to either agent and defined as at risk. 1934-1949, 33 bladder tumours diagnosed, 22 carcinomas, 11 papillomas in 30 individuals, 15/30 known to have had definite exposure to antioxidants from 1400 defined as at risk, 10 carcinomas found (8.5 expected) and 6 papillomas found (1.4 expected)	Veys (1969)

Table 12/contd

Compound	Compound No.	Exposure	Disease/Number affected	References
Naphthylamines contd				
N-phenyl-2-naphthylamine (contaminated by 15-50 mg/kg 2-naphthylamine)	(60)	rubber tyre factory	1946-1960, 3301, out of workforce of 4177 exposed, 9 cases of bladder tumour (10 expected) in total workforce 1331/4177, which 1088 known to be exposed were followed up for >20 years (1946-1970), 3 cases of bladder cancer found (5.5 expected)	Veys (1973)
chlornaphazine	(63)	used to treat polycythemia	14 cases of bladder cancer reported by 1970; 10/61 treated in one study	Thiede et al. (1964) Videbaek (1964) Thiede & Christensen (1969) Laursen (1970)

[a] cited in IARC (1982a)

[b] cited in IARC (1978)

[c] cited in IARC (1982b)

[d] cited in Fishbein (1979)

Footnote: The information contained in this Table is supplementary to that contained in the text.

were associated with the synthesis of the precursors *o*-toluidine and 4,4′-methylenebis(2-methylaniline). Two deaths were associated with the part of the plant where synthesis of the two dyes occurred, where exposures to *o*-toluidine, 4,4′-methylenebis (2-methylaniline) and *o*-nitrotoluene (magenta ABN manufacture) and 2,5-diaminotoluene (35), aniline and *o*-aminoazotoluene (70) (reaction intermediate) (safranine-T manufacture) were possible. In the production of magenta (Basic Fuchsin) (75)*, a process also associated with an increased risk of bladder cancer, *o*-toluidine, *p*-toluidine and aniline are used but not 4,4′-methylenebis-(2-methylaniline) (Rubino et al., 1979).

IARC has evaluated several anilines and phenylenediamines for carcinogenic risk to humans (IARC, 1978, 1982a). It is considered that *o*-toluidine is probably carcinogenic to humans (IARC, 1982a). Although there are some reports concerning human cancer risk/lack of risk associated with both aniline and *p*-chloro-*o*-toluidine, the available information is considered to be insufficient for an evaluation to be made of their carcinogenicity to humans (IARC, 1978, 1982a). There have been many case reports of renal pelvic cancer associated with abuse of analgesic mixtures containing phenacetin (19). In a hospital-based study, patients with interstitial nephritis associated with analgesic abuse had a statistically significant higher prevalence of transitional-cell carcinoma than patients with interstitial nephritis not associated with analgesic abuse (4/48 versus 0/98). Also phenacetin *per se* is regarded as probably carcinogenic to humans (IARC, 1982c).

6.4 Benzidines

6.4.1 Pharmacokinetics and metabolism

6.4.1.1 Animals: Absorption The identification of benzidine (45) and its metabolites in the urine of dogs treated orally, dermally or by aerosol inhalation indicates that some absorption occurs by all these routes (IARC, 1982b). When radiolabelled [^{14}C]-Direct Black 38 was applied dermally to rabbits 91% of the radioactivity was recovered in the urine (EPA, 1979).

Distribution Pharmacokinetic studies with ^{14}C-labelled benzidine have been carried out in rats, dogs and monkeys. In all 3 species the ^{14}C label of 0.2 mg/kg benzidine was rapidly transferred to the excretory organs: liver, gastrointestinal tract, kidney and bladder. Significant amounts were also distributed to the lung (IARC, 1982b).

Metabolism A summary of biotransformation products of benzidine in various mammalian species is shown in Table 13. It can be seen that considerable metabolic differences occur between species. An interesting difference is the apparent inability of dogs to acetylate (or otherwise conjugate) the amino groups of either benzidine or its congeners. Experiments with rats, female dogs and rhesus monkeys have demonstrated the reductive cleavage of a number of benzidine-based dyes to benzidine (Haley, 1982). The metabolic pathways in the Rhesus monkey closely resemble those

*Commercial magenta, known also as Basic Fuchsin or Basic Violet 14, is a mixture of three closely related compounds (see IARC, 1974) in which rosanilin (75) predominates. Magenta ABN is not a component of Basic Fuchsin and is also known as New Fuchsin or Basic Violet 2.

Table 13 Summary of indentified urinary metabolites of benzidine (45) in various species

Metabolite	Species					
	Mouse	Rat	Guinea Pig	Rabbit	Dog	Monkey
3-Hydroxybenzidine				X	X	
3-Hydroxybenzidine hydrogen sulphate				X	X	
Monoacetylbenzidine		X		X		X
Monoacetylated 3-hydroxyglucuronide	X					
Benzidine-*N*-glucuronides (unspecified)	X			X		
Benzidine-*N*-hydrogen sulphate	X					
4'-Amino-4-diphenyl sulphamate		X		X		
4'-Acetamido-4-diphenyl sulphamate		X		X		
4,4'-Diamino-3-diphenyl glucuronide	X			X	X	
4,4'-Diamino-3-diphenyl hydrogen sulphate		X			X	
4'-Acetamido-4-aminodiphenyl *N*-glucuronide			X			
4'-Acetamido-4-amino-3-diphenyl hydrogen sulphate	X	X	X	X		

From Haley (1975,1982), EPA (1979)

in humans. Levels of benzidine in the urine were much greater than those present as impurities in the administered dyes. Lysis of the azo linkages by gut microflora is probably the principal means of producing benzidine from benzidine-based dyes (EPA, 1979). 4-Aminobiphenyl (72) has been reported as a urinary metabolite of Direct Black 38 in guinea pigs (NIOSH, 1980).

Extensive metabolism of 3,3′-dichlorobenzidine (50) is presumed to occur in the dog since although only ~2% of an i.p. dose was excreted in 15 days as the parent compound, in another experiment >95% of radiolabel following i.v. administration of [^{14}C]-3,3′-dichlorobenzidine was excreted in 7 days (Haley, 1975; EPA, 1979). However, no metabolites of 3,3′-dichlorobenzidine have been identified in several experimental animal studies. Since the experimental conditions used were similar to those used in studies on benzidine metabolism, it was concluded that 3,3′-dichlorobenzidine is metabolized differently to benzidine (EPA, 1979). A number of animal studies (in rats, rabbits and monkeys) on the metabolic fate of 3,3′-dichlorobenzidine-based pigments have failed to provide unequivocal evidence that they are broken down to release free 3,3′-dichlorobenzidine (EPA, 1979; Haley, 1982). The failure to detect reductive cleavage products from the 3,3′-dichlorobenzidine-pigments studied may be due to the presence of the substituent group (RC=C(CH_3)-OH) adjacent to the azo-group in all the pigments which have been tested. This substituent group is capable of keto-enol tautomerism, the keto form of which lacks an azo-linkage and would therefore not be susceptible to enzymatic reduction (EPA, 1979). If correct, this hypothesis may also account for the reported lack of carcinogenicity of some 3,3′-dichlorobenzidine pigments tested in animals.

Following intraperitoneal injection of 3,3′-dimethylbenzidine (46) and 3,3′-dimethoxybenzidine (48) in dogs some metabolism to sulphate esters including the 5-hydroxy sulphate ester of 3,3′-dimethylbenzidine occurred, although unmetabolized parent compound was also observed in the urine in both cases (Haley, 1982). Several 3,3′-dimethylbenzidine-based dyes are reductively cleaved to the parent amine by female dogs. Similarly, 3,3′-dimethoxybenzidine-based dyes are cleaved to the parent amine by female dogs and rats (Haley, 1982). In contrast, no evidence of reductive metabolism of the 3,3′-dimethylbenzidine-based pigment, Pigment Yellow 16, was found in rats, a finding consistent with the 3,3′-dichlorobenzidine-based pigments previously mentioned (this pigment also has a group adjacent to the azo linkage capable of keto-enol tautomerism).

Elimination and excretion The blood half-lives of radiolabelled benzidine in the rat and dog have been reported to be 68 and 88 h respectively (Haley, 1975). Dogs and monkeys excreted 65–70% benzidine (as radiolabel) in urine and 30–35% in faeces. In contrast, rats excrete approximately 80% in faeces (probably via the bile) (Haley, 1975, 1982). The weekly excretion of a dose of 0.2 mg/kg of benzidine in the rat, dog and monkey were 97%, 96% and 83% respectively (Haley, 1975).

Little quantitative information is available regarding the elimination of benzidine-based dyes and benzidine congener-based dyes. Benzidine has been detected in the urine of mice, rats, hamsters, dogs and monkeys exposed to a number of benzidine-based dyes. Following administration of 100 mg/kg of 6 different benzidine-based Direct dyes to female dogs, the amount of benzidine excreted in the urine varied from 320-1,675 μg (after 3 days) (NIOSH, 1980). Following oral administration of 10 mg/kg Direct Black 38 (76) to hamsters, a total of 10% of the dye was excreted as benzidine and benzidine conjugates in the urine. Analysis of 24 h urine samples from mice and rats administered Direct Blue 6 (77) in the diet showed a clear-cut relationship between the concentration of dyestuff consumed and the amount of benzidine excreted.

Rats and dogs excreted 3,3′-dichlorobenzidine mainly via the faeces, the dog faecal excretion being 10 times greater than urinary. Neither route of excretion was preferred by the monkey (Haley, 1975, 1982). The weekly excretion values for 3,3′-dichlorobenzidine (i.v. administration, 0.2 mg/kg) in rats, dogs and monkeys were 98%, 97% and 89% administered dose respectively (Haley, 1975).

Following i.p. administration of 70–100 mg/kg of 3,3′-dimethylbenzidine to dogs, 4% of free 3,3′-dimethylbenzidine and 40% of a metabolite (probably the 5-hydroxy ethereal sulphate derivative) were recovered in the urine within 3 days (Fishbein, 1979).

Following administration of a dose of 1 g of 3,3′-dimethoxybenzidine to dogs, 0.4% of the free compound and about 5% of a metabolite (with properties similar to 3,3′-dihydroxybenzidine) were isolated in the urine (Fishbein, 1979).

6.4.1.2 Humans:Absorption Benzidine (45), 3,3′-dichlorobenzidine (50), 3,3′-dimethylbenzidine (46), 3,3′-dimethoxybenzidine (48), and benzidine-based dyes may enter the body by percutaneous absorption, ingestion or inhalation (Fishbein, 1980; IARC, 1982b). Percutaneous absorption appears to be the primary route following occupational exposure. Following application of 100 mg benzidine to the skin <0.02 mg benzidine and metabolites were detected in the urine (IARC, 1982b). Workers who perspire freely and have wet skin have higher levels of benzidine in their urine (Meigs et al., 1954). Large particle size and non-volatility make 3,3′-dichlorobenzidine less of an inhalation hazard than benzidine (Haley, 1982). In one worker drenched with a slurry of 3,3′-dichlorobenzidine in water, urinary excretion was increased approximately 10 times (Meigs et al., 1954).

Distribution Little information is available on the tissue distribution of benzidine and its congeners in man. However, it has been reported that accumulation does not take place (Haley, 1982).

Metabolism A variety of biotransformation products of benzidine and congeners have been found in human urine. Exposure of plant workers to an unknown quantity of benzidine resulted in urinary excretion of free benzidine (4–6%), monoacetylbenzidine (1–5%), diacetylbenzidine (5–10%) and 3-hydroxybenzidine conjugates (80–90%) (IARC, 1982b). *N*-hydroxy-*N*-acetylaminobenzidine and *N*-hydroxybenzidine have also been reported in the urine after ingestion of benzidine (200 mg) (Haley, 1982; Kiese, 1974). Kiese (1974) also reported 3,3′-dihydroxybenzidine as a metabolite from an *in vitro* study using human liver microsomes.

Benzidine and its metabolic derivatives, notably *N*-acetyl-derivatives, have also been detected in the urine of workers exposed to benzidine-based dyes (EPA, 1979; NIOSH, 1980; IARC, 1982b). Urinary benzidine levels found in seven workers potentially exposed to benzidine-based dyes were much higher than could be attributed to residual benzidine present as a contaminant in the dyes, thus demonstrating a metabolic release (via reductive cleavage of the azo linkages) of benzidine from the dyes. Studies using *E coli* isolated from humans have shown that Direct Black 38 (76) may be reduced to free benzidine (EPA, 1979; NIOSH, 1980). A benzidine-albumin complex has been found in the blood serum of workers exposed to direct dyes. The amount of this complex was dependent on the extent and duration of exposure (NIOSH, 1980).

There are no reports in the literature regarding the biotransformation of 3,3′-dichlorobenzidine although the unmetabolized amine has been detected in the

urine of exposed workers. Workers handling Pigment Yellow 13 excreted 3,3′-dichlorobenzidine in their urine (Akiyama, 1970). Most studies, however, indicate that 3,3′-dichlorobenzidine-based pigments are poorly, if at all metabolized to the parent amine (Haley, 1982).

Exposure to 3,3′-dimethylbenzidine results in the urinary excretion of free 3,3′-dimethylbenzidine, its diacetyl derivative and 5-hydroxy-3,3′-dimethylbenzidine, and conjugates (Haley, 1982; EPA, 1979). Although no reports of human metabolism of 3,3′-dimethylbenzidine-based dyes could be found in the literature, it is possible that, as observed in mammalian species, intestinal bacteria could release 3,3′-dimethylbenzidine from 3,3′-dimethylbenzidine-based dyes (EPA, 1979).

The biotransformation of 3,3′-dimethoxybenzidine has not been reported in humans although the unmetabolized amine has been identified in urine. 3,3′-Dimethoxybenzidine has been found in the urine of a worker exposed to 3,3′-dimethoxybenzidine-based dyes indicating that reductive cleavage does occur (Haley, 1982).

Excretion Haley (1975) reported that only 1% of a single oral dose of benzidine (100 mg) was recovered in the urine indicating faecal excretion and/or cumulation in body tissues. In one dye manufacturing facility, four workers were monitored for exposure to benzidine-based dyes. The total airborne particulate material exposures of these workers were 4.3, 5.2, 11.7 and 17.4 mg/m^3 respectively. The corresponding urinary concentrations of benzidine averaged 52, 11, 10 and 112 ppb respectively. 590, 248 and 22 ppb monoacetylbenzidine were detected in the urine samples containing 112, 52 and 11 ppb benzidine (IARC, 1982b). Benzidine conjugates other than the mono- and diacetyl derivatives were detected in the urine of three of these workers (NIOSH, 1980). In another factory manufacturing Direct Black 38 (76) and Direct Blue 2 (both benzidine-based dyes), urinary concentrations of benzidine in seven potentially exposed workers ranged from below the detection limit to 0.039 mg/litre (39 ppb). The total airborne particulate material ranged from 1 to 4 mg/m^3.

Quantitative data concerning excretion of benzidine, congeners and related dyes in human beings are not available. Both 3,3′-dichlorobenzidine and 3,3′-dimethoxybenzidine have been detected in the urine of workers exposed to either the parent compound or related dyes or pigments. In contrast, 3,3′-dimethylbenzidine has been detected in human urine following exposure to the parent amine only. No reports on analysis of faeces from workers exposed to benzidine, benzidine congeners or benzidine-based dyes appear in the literature.

6.4.2 Short-term tests

Table 14 summarizes data on mutagenicity and other short-term assays for genotoxicity of benzidines and benzidine-based dyes. The criteria for inclusion under a given type of test are outlined in Section 6.3.2.

6.4.3 Carcinogenicity in experimental animals

IARC has determined that there is sufficient evidence for carcinogenicity in experimental animals for benzidine (45), 3,3′-dichlorobenzidine (50), 3,3′-dimethylbenzidine (46) and 3,3′-dimethoxybenzidine (48) (IARC, 1972, 1982c).

Table 14 Short-term tests: benzidines and benzidine dyes

Compound	Compound No.	Positive results				Negative results			
		DNA damage	Mutagenicity	Chromosome anomalies	Other	DNA damage	Mutagenicity	Chromosome anomalies	Other
Benzidines									
benzidine[a,b]	(45)	X	X	X	X_{CT}	X			
3,3′-diaminobenzidine[c]	(49)		X						
3,3′-dichlorobenzidine[a,b]	(50)	X	X		X_{CT}				
2,2′,5,5′-tetrachloro-benzidine[d]	(52)		X						
3,3′-dimethylbenzidine[c,e,f]	(46)	X	X		X_{CT}				
3,3′-dimethoxybenzidine[a]	(48)	X	X		X_{CT}	X			
N-acetylbenzidine[b]			X						
N-OH-*N*,*N*′-diacetylbenzidine[b]			X						
Benzidine-based dyes									
Acid Orange 45[c]			X						
Acid Red 85[c]							X		
Chlorazol Violet N[c]			X						
Direct Black 38[a,b]	(76)		X						
Direct Red 28[e,f]			X			X	X		
Direct Violet 1[f]			X						

Table 14/contd

Compound	Compound No.	Positive results				Negative results			
		DNA damage	Mutagenicity	Chromosome anomalies	Other	DNA damage	Mutagenicity	Chromosome anomalies	Other
3,3′-Dimethylbenzidine-based dyes									
Direct Blue 14[c,f]	(80)		X				X	X	
Direct Blue 53[c]	(79)					X	X		
Benzopurpurene 4B[c]			X						
2,2′-Dimethylbenzidine-based dyes									
Acid Red 111[c]							X		
Acid Red 114[c]							X		
3,3′-Dimethoxybenzidine-based dyes									
Direct Blue 1[c]							X		
3,3′-Dichlorobenzidine-based dyes									
Pigment Yellow 12[c]			X				X		
Pigment Orange 13[c]							X		

Footnote: Structures of above compounds not listed in Figure 2 can be found in Combes & Haveland-Smith (1982)

From:

[a] IARC (1982c)

[b] IARC (1982b)

[c] Combes & Haveland-Smith (1982)

[d] IARC (1982a)

[e] Martin et al. (1978)

[f] EPA (1979)

Key:
CT=cell transformation

Studies have also been reported on the 3,3′-diamino and 2,2′-disulphonic acid derivatives of benzidine (Helmes et al., 1984), but have not been evaluated by IARC. Evidence for carcinogenicity in experimental animals was determined as sufficient for the benzidine-based dyes Direct Black 38 (76) and Direct Blue 6 (77) and as limited for Direct Brown 95 (78). Studies on the 3,3′-dichlorobenzidine-based pigments, Pigment Yellow 12 and Pigment Yellow 83 have also been reported (EPA, 1979) but have not been evaluated by IARC. The 3,3′-dimethylbenzidine-based dyes Direct Blue 14 (80) and Direct Blue 53 (79) have also been evaluated by IARC (1975) but sufficient evidence for carcinogenicity was only available for Direct Blue 14. It is interesting to note that the onset of tumour formation in both rats and mice administered Direct Black 38, Direct Brown 95 and Direct Blue 6 was more rapid than when benzidine was administered alone. This suggests that the parent dye (or a metabolite other than benzidine) is carcinogenic and that carcinogenicity is not solely dependent on the presence of benzidine (as an impurity or metabolite) *per se* (NIOSH, 1980). In a recent study of azo dyes to determine priorities for carcinogenicity testing by the National Cancer Institute, the priority selections contained two benzidine-based dyes (Acid Red 85 and Direct Orange 1) and one 2,2′-dimethylbenzidine-based dye (Acid Red 111) (Helmes et al., 1984).

6.4.4 Effects in humans

6.4.4.1 Systemic Exposure to benzidine (45) has been shown to produce a spectrum of lesions of the epithelium of the urinary bladder which may precede appearance of malignancy. These lesions include hyperaemia inflammation and papillomata (both sessile and pedunculated). The development of such lesions may be accompanied by haematuria and/or impaired micturition. An increase in the number of sister chromatid exchanges, of questionable significance, has been reported in peripheral blood lymphocytes of 15 people occupationally exposed to benzidine (IARC, 1982b).

6.4.4.2 Dermatitis Benzidines are often present in organic dyes where they have been documented as allergens (Foussereau et al., 1982). Usually the exact diagnosis of allergy is difficult in factories where dyes are synthesized or used because of the large number of products involved. Occupational dermatitis has been reported among dye workers exposed to 3,3′-dichlorobenzidene (50) (Gerarde & Gerarde, 1974) and the benzidine-based dye Acid Red 85 (Pedersen, 1982).

As benzidine is usually not a component in standard patch tests its allergenic potential is not completely known. However, the results of one study from Spain are summarized in Table 9 (Grimalt & Romaguera, 1981). The only real common possible causative factor in all the patients reacting to benzidine was clothing and shoes. Also, rivers in the study area received residual water drained from local textile industries and therefore the river water, which was used to irrigate large fields where vegetables were cultivated, may have become contaminated by azodyes. It is possible that the suspected presence of contaminating azodyes in raw vegetables may have had an additional significance for patients who were primarily sensitized by contact.

6.4.4.3 Carcinogenicity An association between industrial exposure to benzidine (45) and bladder cancer has been recognized since the early decades of the 20th century

(see Table 12). Primary tumours at sites other than the bladder have been noted in several of the series of workers exposed to benzidine. Historical reviews (Haley, 1975) have reported that the worldwide spread of bladder cancer in people exposed to benzidine has followed the international spread of the dyestuffs industry. In addition to an association with bladder cancer, benzidine alone, or in combination with 2-naphthylamine (58), has also been implicated in an elevated risk for pancreatic cancers (Mancuso & El-Attar, 1967), cancers of the prostate, stomach and rectum (Fishbein, 1979) and in second primary cancers of the liver, gall bladder, bile duct, large intestine and lung, subsequent to initial diagnosis of tumours of the genito-urinary organs (Morinaga et al., 1982). On the basis of epidemiological studies IARC (1982b) have concluded that sufficient evidence exists to consider benzidine and its dihydrochloride as carcinogenic in man.

There is some evidence to suggest that benzidine-exposed workers with low serum properdin, an important immunoprotein, are more susceptible to bladder cancer (Radomski, 1979). Also at the biochemical level, benzidine has been shown to increase β-glucuronidase activity in exposed workers. After cessation of exposure this activity does not return to normal (Haley, 1982).

3,3′-Dimethylbenzidine (46) was reviewed by IARC (1972). No case reports of cancer in workers exposed soley to 3,3′-dimethylbenzidine have been reported.

According to IARC (1982b) no case reports are known in which 3,3′-dichlorobenzidine (50) has been associated with the occurrence of cancer in man. However, because 3,3′-dichlorobenzidine and benzidine are often made in the same plant, it cannot be excluded that 3,3′-dichlorobenzidine may have contributed to the incidence of bladder cancer attributed to benzidine. IARC (1982b) concluded that epidemiological data were inadequate to evaluate the carcinogenicity of 3,3′-dichlorobenzidine *per se*, but nevertheless should be regarded as probably carcinogenic to humans.

3,3′-Dimethoxybenzidine (48) was reviewed by IARC (1974). No epidemiological data on the occurrence of cancer in workers exposed to this compound alone appear in the literature. 3,3′-Dimethoxybenzidine has also been prepared in the same plants as benzidine and again may have contributed to the increased bladder cancer risk among benzidine workers and again should be regarded as probably carcinogenic to humans.

Several epidemiological studies of dye users and dye workers suggest that there may be excess mortality from bladder cancer in people exposed to benzidine-based dyes. Two studies, one Japanese (Yoshida et al., 1971) and one Russian (Genin, 1977) (see Table 12), referred to five benzidine-based dyes, Direct Black 38 (76), Direct Green 1, Direct Red 17, Direct Red 28 and Direct Blue 2, as being the most widely used and therefore the most suspect in the etiology of increased bladder cancer in dye workers. Workers in the Russian study (Genin, 1977) were also exposed to two 3,3′-dimethoxybenzidine-based dyes, Direct Blue 15 and Direct Blue 218.

Although current epidemiological data are inadequate to evaluate the carcinogenicity of any individual benzidine-based dyes to humans, IARC (1982b) have concluded that evidence exists to indicate that occupational exposure to either Direct Black 38, Direct Blue 6 (77) or Direct Brown 95 (78) presents a carcinogenic risk.

Benzidine-based dyes have also been reported to contain residual amounts of benzidine and 4-aminobiphenyl (72). Existing epidemiological evidence (Table 12) has shown that occupational exposure to 4-aminobiphenyl has been strongly associated with bladder cancer. It has been reported that the carcinogenic potency of 4-aminobiphenyl in man is at least equal to, and possibly even greater than, 2-naphthylamine (NIOSH, 1980).

6.5 4,4′-Diaminodiphenylmethanes

6.5.1 Pharmacokinetics and metabolism

6.5.1.1 Animals Urinary excretion of the parent amine or its metabolites has demonstrated absorption of 4,4′-methylenebis(2-chloroaniline) (54) in rats via p.o. or p.c. routes (Farmer et al., 1981; Manis et al., 1984) and in dogs via p.c. routes (Manis et al., 1984). Following skin application of 4,4′-methylenebis (2-chloroaniline) to dogs, approximately 2.4% of the dose only was estimated to have been absorbed via the skin (Manis et al., 1984). Similarly, only 1.5% of a p.c. dose of 4,4′-methylenebis(2-chloroaniline) was reported to be absorbed in rats (HSE, 1983).

Relatively high calculated octanol/water partition coefficients for 4,4′-methylenebis (2-chloroaniline) (log P 3.5) and 4,4′-methylenebis(2-methylaniline) (55) (log P 3.92) indicate that significant tissue accumulation might be expected (Radding et al., 1977). Similarly, following a bolus i.v. injection in the dog the elimination phase half-time for 4,4′-methylenebis(2-chloroaniline) in the blood was 0.7 h and the apparent volume of distribution was 244 litres, indicating rapid metabolism and/or extensive accumulation in other tissues.

4,4′-Methylenebis(2-chloroaniline) has been detected primarily in the liver, kidney, fat and lung following administration to dogs (Manis et al., 1984) with lesser amounts in the urinary bladder (HSE, 1983); and in the small intestine, liver, fat, lung, kidney adrenals and skin in rats (Farmer et al., 1981; HSE, 1983). Following i.v. administration of [^{14}C]-4,4′-methylenebis(2-chloroaniline) to rats, hepatic radioactivity was associated mainly with the nuclear and cytosolic fractions (HSE, 1983). Similarly, following [^{3}H] 4,4′-methylenebis(*N,N*-dimethylaniline) injection in rats, radioactivity was found bound to liver nucleic acids (IARC, 1982a).

Following i.p. administration of methylenedianiline to rats, 25% of the dose was excreted in the bile within 24 h as three or four unidentified metabolites and little unchanged amine (Kopelman et al., 1966b). Similarly, several studies have demonstrated fairly rapid metabolism of 4,4′-methylenebis(2-chloroaniline) with extensive excretion of metabolites but little parent amine observed in the urine and bile, following administration to rats and dogs (Farmer et al., 1981; HSE, 1983; Manis et al., 1984).

Metabolites of 4,4′-methylenebis(2-chloroaniline) have not been extensively characterized in animals. An early report identified the major urinary metabolite following oral administration to dogs as the 5-hydroxy-derivative. Recently, however, following i.v. administration to dogs, a sulphate conjugate was identified as the major (85%) urinary metabolite. Fifteen metabolites have been reported following oral dosing of rats, with some evidence for *N*-acetyl- and *N,N′*-diacetyl-derivatives, cleavage of the methylene bridge, and *O*-glucuronide and *O*-sulphate conjugation (HSE, 1983). A deficiency of dietary protein has been reported to cause a reduction in the excretion of metabolites (HSE, 1983) and, in chronic dosing, excretion of unmetabolized 4,4′-methylenebis(2-chloroaniline) increases with time (Manis et al., 1984).

Approximately 30–40% of a dose of 4,4′-methylenebis(2-chloroaniline) administered p.o. or i.p. to rats appeared in the urine, and 46% of a bolus i.v. dose in dogs was excreted in the 24 h urine. However, only 3–6% and 0.25% respectively was unmetabolized amine. In the latter case 32% of the dose was recovered from the gall bladder bile at 24 h with no unmetabolized 4,4′-methylenebis(2-chloroaniline)

present (Farmer et al., 1981; Manis et al., 1984). Following p.c. application of 4,4′-methylenebis(2-chloroaniline) to dogs, 1.3% of the administered dose was recovered in the 24 h urine (0.005% dose as the unchanged amine) and 0.6% was recovered in gall bladder bile.

6.5.1.2 Humans Percutaneous absorption has been proposed to be the most likely route of exposure to 4,4′-methylenebis(2-chloroaniline) (54) in the human populations studied (Linch et al., 1971; Manis et al., 1984). *In vitro* experiments with human skin have demonstrated that 4,4′-methylenebis(2-chloroaniline) may be rapidly absorbed and transported through the skin (HSE, 1983). Absorption via the oral route with rapid excretion of ingested compound has also been reported (HSE, 1983).

4,4′-Methylenebis(2-chloroaniline) has been detected in erythrocytes in humans. This may represent at least one cell type with potential for storage of the compound (Manis et al., 1984).

Although Glowinski et al. (1978) observed that 4,4′-methylenebis(2-chloroaniline) is acetylated *in vitro* by a genetically determined polymorphic *N*-acetyltransferase isolated from human liver, no acetyl-conjugates have been found in human urine. As yet no metabolites of 4,4′-methylenebis(2-chloroaniline) have been positively identified in human urine following exposure to the amine, although an *N*-glucuronide conjugate has been tentatively identified.

4,4′-Methylenebis(2-chloroaniline) has been identified in the urine of exposed workers (Linch et al., 1971; ACGIH, 1980; Lower, 1982), pre-school children resident in an area contaminated by the chemical from a manufacturing plant, and families of employees exposed at the same plant (Manis et al., 1984). In one study of employees from the plant causing contamination in its vicinity (see Section 4.1.3 for details) urinary excretion levels of up to 49 mg/litre were found. If, as seems reasonable from animal experiments, it is assumed that 4,4′-methylenebis(2-chloroaniline) is rapidly metabolized so that only a few per cent of the absorbed dose would appear unchanged in the urine, such levels would be equivalent to exposures of near gram quantities for some individuals (Lower, 1982). In another plant, urinary excretion levels as high as 25 mg/litre were found early in the compound's manufacturing history, although in a later twelve-month survey involving 429 specimens from employees at the same plant, the maximum urinary level of 4,4′-methylenebis(2-chloroaniline) found was 6.72 mg/litre. In particular the average urinary excretion levels in four employees who were monitored during investigations into atmospheric and surface contamination at the plant (see Section 4.2.3 for details) and who were employed in the pelletizing and packaging operation were found to be 0.07, 0.25, 0.96 and 1.5 mg/litre (variation 0.04−3.8 mg/litre). The results indicate relative differences in aromatic amine metabolism between individuals. Exposed workers could be classified into 3 categories depending on their urinary levels of 4,4′-methylenebis(2-chloroaniline): non-excretors (0.5 mg/litre or less), intermediates (0.5−2 mg/litre) and excretors (greater than 2 mg/litre). Excretors were found to require more than 1 month for urinary excretion levels of 4,4′-methylenebis(2-chloroaniline) to decline from >1 mg/litre to <0.04 mg/litre whereas non-excretors required 1 week. It was not possible to establish a relationship between exposure dose and urinary excretion rate but no acute toxicity or evidence for chronic disease was demonstrated (Linch et al., 1971). A urinary level of 4,4′-methylenebis(2-chloroaniline) of 3.6 mg/litre was found subsequent to a worker being splashed in the face with molten material (see Section 6.5.4 for details) (Hosein & Van Roosmalen, 1978).

Among a group of 27 workers producing 4,4′-methylenedianiline (53), the percentage of urine samples found to contain 4,4′-methylenedianiline decreased from about 15 per cent in 1970 to 0.1 per cent in 1980. Levels also decreased from >200 μg/l to >20μg/l IARC, 1986). In contrast, no 4,4′-methylenedianiline was detected in the urine of a group of exposed employees despite a 12% incidence of toxic hepatitis believed to be associated with the diamine (McGill & Motto, 1974).

6.5.2 Short-term tests

Table 15 summarizes data on mutagenicity and other short-term assays for genotoxicity of 4,4′-aminodiphenylmethanes. The criteria for inclusion under a given type of test are outlined in Section 6.3.2.

6.5.3 Carcinogenicity in experimental animals

IARC has determined that there is sufficient evidence for carcinogenicity in experimental animals for 4,4′-methylenedianiline (53), 4,4′-methylenebis-(2-methylaniline) (55) and 4,4′-methylenebis(2-chloroaniline) (54) and limited evidence for 4,4′-methylenebis(*N*,*N*-dimethylaniline) (56) (IARC, 1974, 1982a, 1986).

6.5.4 Effects in humans

6.5.4.1 Systemic 4,4′-Methylenedianiline (53) is hepatotoxic in humans after acute exposure, both orally and following atmospheric contamination. Exposure may be via respiratory or percutaneous routes (Kopelman et al., 1966a; McGill & Motto, 1974; Williams et al., 1974; Brooks et al., 1979). Industrial poisoning has been reported following exposure to air concentrations of 0.04-3 mg/m^3 (0.005−0.373 ppm). Conversely, exposure to air concentrations as high as 29 mg/m^3 (3.8 ppm) (TWA 0.24−3.2 mg/m^3 (0.03−0.4 ppm)) has been reported with no ill-effect (ACGIH, 1980).

Kopelman et al. (1966a) reported an outbreak of jaundice in the Epping (U.K.) area following the ingestion of wholemeal bread made from flour accidentally contaminated in transit with 4,4′-methylenedianiline. Eighty-four people were known to have been affected. Three major clinical courses of the disease were apparent:

(i) in the most common form there was an acute onset of severe intermittent pain in the upper abdomen and lower chest which lasted from 24 to 36 hours, followed by some improvement then, after 4−5 days, pyrexia, 'flu-like symptoms and increasing jaundice with an enlarged and tender liver;

(ii) initial symptoms were vague with some upper abdominal discomfort, followed about one week later by pyrexia, general aches and increasing jaundice which was more persistent than in the group experiencing more severe initial symptoms;

(iii) some elderly patients experienced minimal early symptoms but presented with severe jaundice.

Liver biopsy specimens showed portal inflammation, eosinophilic infiltration, cholangitis, cholestasis and hepatocellular damage (Kopelman et al., 1966b).

Table 15 Short-term tests: 4,4′-diaminodiphenymethanes

Compound	Compound No.	Positive results				Negative results			
		DNA damage	Mutagenicity	Chromosome anomalies	Other	DNA damage	Mutagenicity	Chromosome anomalies	Other
4,4′-methylenedianiline[a]	(53)	X	X	X			X	X	
4,4′-methylenebis-(2-chloroaniline)[c]	(54)	X	X	X	X_{CT}	X	X	X	X_{CT}
4,4′-methylenebis(*N*,*N*-dimethylaniline)[b]	(56)	X	X		X_{CT}		X		
Structurally related compounds:									
auramine[d]	(74)	X	X			X	X		X_{CT}
magenta[d] (Basic Fuchsin)	(75)	X				X	X	X	X_{CT}
magenta ABN[d] (New Fuchsin)						X			

From:

[a] IARC (1986)

[b] IARC (1982a)

[c] HSE (1983)

[d] Combes & Haveland-Smith (1982)

Key:

CT=cell transformation

Roy et al. (1985) recently reported toxic optic neuritis with severe prolonged visual dysfunction in addition to toxic hepatitis following accidental ingestion of 4,4′-methylenedianiline in potassium carbonate and γ-butyrolactone. Eighteen months after the incident visual function was still seriously impaired and liver function tests remained disturbed.

Being a solid with a low vapour pressure, 4,4′-methylenedianiline might not be expected to be a significant inhalation hazard, but grinding or heating processes may result in the formation of dusts or vapours which may be especially dangerous (Moore, 1978).

Absorption of 4,4′-methylenedianiline may follow repeated or prolonged skin contact especially when a solution is handled (Moore, 1978). In at least one report of industrial poisoning, hepato-toxicity was almost certainly due to cutaneous exposure rather than inhalation (McGill & Motto, 1974, Table 12), since adverse effects continued to be evident even when atmospheric contamination was reduced considerably, and symptoms only occurred in those manually mixing a resin, with the diamine as a hardener, wearing only cotton gloves for protection. The disease incidence continued despite the use of helmets with separate air supplies, until rubber gloves and eventually a fully enclosed automated blending system were introduced.

One report of poisoning following occupational exposure to 4,4′-methylenedianiline in which exposure may have been via ingestion, percutaneous or respiratory routes describes, in conjunction with a rash on the exposed skin and hepatotoxicity, myocardiopathy consistent with lateral wall injury and ischaemia (Brooks et al., 1979).

If absorbed in sufficient quantity, 4,4′-methylenebis(2-chloroaniline) (53) may produce cyanogenic effects. However, the cyanosis anaemia syndrome was not observed in a group of exposed employees despite significant urinary excretion of the diamine (up to 25 mg/litre) (Linch et al., 1971). Absorption was thought to have been primarily via the skin, since the maximum possible amount of diamine inhaled could not have accounted for the urinary levels found, even if complete pulmonary absorption had occurred (see Section 4.2.3 for discussion). 4,4′-Methylenebis (2-chloroaniline) has been implicated as a causative agent in the development of urinary frequency with haematuria in 2 people occupationally exposed. The route of exposure was believed to be largely inhalation of dust and heated vapour and there was concomitant exposure to other materials including toluene diisocyanate-containing- and polyester- and polyether-resins (Mastromatteo, 1965). An employee who was accidentally splashed in the face with hot liquid 4,4′-methylenebis(2-chloro-aniline) developed conjunctivitis within 1−2 h. Some of the liquid was also swallowed, resulting in "stomach sickness" shortly after the incident (Hosein & Van Roosmalen, 1978).

6.5.4.2 Dermatitis 4,4′-Methylenedianiline which is used for curing both epoxy and urethane resins is a potent allergic sensitizer (Agrup & Fregert, 1969; Breit, 1969) and also stains skin and nails yellow. Of 31 patients with epoxy dermatitis investigated by Behrbohm et al. (1975), 3 reacted only to hardeners of which two were based on aromatic amino-polyamides and one contained 4,4′-methylenedianiline.

Occupational photosensitivity to 4,4′-methylenedianiline has been described (LeVine, 1983).

6.5.4.3 Carcinogenicity Although there is no evidence available regarding the

carcinogenicity of 4,4′-methylenedianiline in humans, it is considered by both NIOSH and OSHA to be a suspected carcinogen (Moore, 1978; Beard & Noe, 1981).

Despite the extent of exposure to (Section 4.2.3) and absorption of (Section 6.5.1.2) 4,4′-methylenebis(2-chloroaniline) demonstrated by Linch et al. (1971), no cytological evidence of bladder cancer was found on periodic examination in the population under study (Table 12). In contrast, Cartwright (1983) reported that interim data from an epidemiological study of a U.K. plant manufacturing 4,4′-methylenebis (2-chloroaniline) indicated a significant excess of bladder cancer. The HSE (1983) concluded that these findings could only be assessed upon completion of the study. The ACGIH (1980) lists 4,4′-methylenebis(2-chloroaniline) as an "industrial substance suspected of carcinogenic potential for man", with the possibility that high exposures would be most likely to cause liver or bladder cancers.

No reports describing carcinogenicity in humans, or any epidemiological studies for 4,4′-methylenebis(*N*,*N*-dimethylaniline) (56) or 4,4′-methylenebis(2-methylaniline) (55) used alone have been identified. Following investigations in a dyestuff factory, however, where employees were exposed to several different aromatic amines, Rubino et al. (1979) (see Section 6.3.4.3 for details) proposed that 4,4′-methylenebis(2-methylaniline) should be considered as a potential cause of urinary bladder cancer in humans.

The manufacture of auramine (74), magenta (75) and magenta ABN, dyes structurally related to 4,4′-methylenebis (*N*,*N*-dimethylaniline), 4,4′-methylenedianiline and 4,4′-methylenebis(2-methylaniline) respectively, have also been associated with an elevated risk of urinary bladder cancer (Case & Pearson, 1954; Case et al., 1954; Rubino et al., 1979, 1982).

6.6 Naphthylamines

6.6.1 Pharmacokinetics and metabolism

6.6.1.1 Animals An early study reported that the bladder epithelium and red blood cells were the only tissues found to retain [^{14}C]-2-naphthylamine (58) following i.p. injection in the rat. Radioactivity was retained in red blood cells for up to 10 weeks. In a later experiment [^{3}H]-2-naphthylamine was found bound to the liver, kidney and spleen following administration to rats. The relative amount of binding in all 3 tissues was cytoplasmic proteins >nuclear proteins >ribosomal RNA (Arcos & Argus, 1968, 1974). Both 1-naphthylamine (57) and 2-naphthylamine, however, have relatively low octanol/water partition coefficients (for 1-naphthylamine log, P = 1.8 (calculated) and 2.15 (experimental) and for 2-naphthylamine is probably similar), therefore neither would be expected to accumulate significantly in body tissue (Radding et al., 1977).

Major urinary metabolites following administration of 1- or 2-naphthylamine to several animal species are their *o*- and *p*-, or *o*-hydroxy-conjugates respectively (IARC, 1974; Radomski, 1979). For example, following oral dosing of 800 mg [^{14}C]-1-naphthylamine to dogs 56% of the urinary radioactivity was isolated as 1-amino-4-naphthylsulphate and 25% as 1-amino-2-naphthylsulphate, the related glucuronide conjugates were also isolated but in much smaller amounts (7.3 and 3.7% respectively). Following a similar experiment with 2-naphthylamine, 88.6% of the urinary radioactivity was recovered as 2-amino-1-naphthylsulphate and 3.7% as the related glucuronide conjugate. The parent amine and small amounts of the *N*-hydroxy-derivatives were also observed for each isomer but the nitroso-derivative

was isolated only following dosing with 2-naphthylamine (Deichman & Radomski, 1969). Earlier experiments had demonstrated that whereas a single high (70 mg/kg b.w.) oral dose of either naphthylamine isomer in dogs led to similar proportions of hydroxylamine and nitroso-derivatives in the urine for each amine, a lower (5 mg/kg b.w.) dose resulted in a similar proportion (0.2%) of the same metabolites from 2-naphthylamine but barely detectable amounts from 1-naphthylamine (IARC, 1974). Bis(2-amino-1-naphthyl)-hydrogen phosphate (1.7%) was isolated from the urine of dogs administered 2-naphthylamine. A similar metabolite was not isolated following dosing with 1-naphthylamine. However, in the latter case 1-amino-2-naphthyldihydrogen phosphate (1.3%) was observed (Deichman & Radomski, 1969). Other metabolites have been found, but not identified, in the urine of various species following administration of either isomer (Radomski, 1979). 2-Amino-1-naphthylmercapturic acid, via *N*-hydroxylation, and 5-hydroxy-6-mercapturic acid, via ring hydroxylation, have also been identified in urine following dosing of several species with 2-naphthylamine. Acetate, sulphate or glucuronide conjugates of the amino group have also been observed.

Rats and rabbits injected with [^{14}C]-2-naphthylamine do not eliminate radioactivity in expired CO_2, indicating that fragmentation of the aromatic ring structure does not occur (Arcos & Argus, 1974).

2-Naphthylamine was detected in the urine of dogs dosed p.o. with [^{14}C]-*N*-phenyl-2-naphythylamine (60) either as a single dose or following pre-treatment with unlabelled compound. No 2-naphthylamine metabolites were recovered. 2-Naphthylamine, however, was not detected as a product of *in vitro* hepatic microsomal metabolism of *N*-phenyl-2-naphthylamine for several mammalian species (Anderson et al., 1982). Chlornaphazine (63) may also be excreted as dealkylated metabolites. Following injection in rats, 2-amino-1-naphthyl hydrogen sulphate and 2-acetamido-6-naphthyl hydrogen sulphate were detected (IARC, 1974).

The urine:faeces excreted radioactivity ratio following administration of labelled 2-naphthylamine has been observed to decrease in the order dog, rabbit, guinea pig, mouse, rat. Of these species the dog and rat excrete the highest proportion of 2-naphthylamine metabolites via the urine and are the most susceptible to 2-naphthylamine-induced bladder cancer (Arcos & Argus, 1974). Within three days of oral administration of [^{14}C]-*N*-phenyl-2-naphthylamine to dogs, 90% of the total radioactivity had been excreted, largely in the faeces (IARC, 1974).

An estimation of urinary levels of *N*-hydroxyarylamines may be complicated by the rapid resorption of metabolites across the bladder wall and the formation of *N*-hydroxylamines in urine from glucuronide conjugates. Young & Kadlubar (1982) have generated a pharmacokinetic model to predict the exposure of the bladder epithelium to urinary *N*-arylhydroxylamines as a function of urine pH, voiding interval and resorption. The model evaluates the ratio between total *N*-arylhydroxylamine exposure in the bladder lumen and *N*-arylhydroxylamine levels in the urine. On consideration of urine pH and frequency of micturition the model correctly predicts the order of susceptibility of different species to 2-naphthylamine-induced bladder cancer, man $\geqslant$dog $>$monkey $>$rat, which may indicate that these are important variables for aromatic amine induced bladder cancer.

6.6.1.2 Humans Both 1-naphthylamine (57) and 2-naphthylamine (58) and *N*-phenyl-2-naphthylamine (60) have been administered orally to humans and absorption has been demonstrated in each case by the presence of the parent amine or metabolites

in the urine. Since urinary excretion of the parent amine and related metabolites has also been observed in exposed workers, inhalation and skin absorption are other possible routes of exposure.

Early 1-naphthylamine workers excreted 10–40 mg 1-naphthylamine/day in their urine. It has been estimated that only approximately 5% of amine-derived substances in the urine is free amine, for both 1- and 2-naphthylamine. This would indicate that the absorbed dose of 1-naphthylamine would have been of the order of 10 mg/kg/day (Radomski, 1979), i.e. possibly more than half a gram for some individuals.

Excretion of *N*-hydroxy-1-naphthylacetamide either free or as a glucuronide conjugate was observed in 4 patients following doses of 250 mg 1-naphthylamine. Similarly, following administration of 2-naphthylamine to hospital patients both the *N*-hydroxy-derivative and bis(2-amino-1-naphthyl) phosphate were identified in the urine (IARC, 1974).

N-Phenyl-2-naphthylamine has been demonstrated to be metabolized to 2-naphthylamine in humans (Kummer & Tordoir, 1975; Moore et al., 1977). The presence of μg quantities of 2-naphthylamine has been reported in the 24 h urine of volunteers who ingested 10–50 mg *N*-phenyl-2-naphthylamine. The *N*-phenyl-2-naphthylamine was contaminated with low levels of 2-naphthylamine but in each case the amount of 2-naphthylamine excreted exceeded the maximum amount ingested by up to 400 times (Kummer & Tordoir, 1975). 2-Naphthylamine was also detected in the urine of workers who had inhaled up to an estimated 40 mg *N*-phenyl-2-naphthylamine. In one inhalation study the amounts excreted (3–8 μg in 24 h) also exceeded the maximum amount absorbed (0.03 μg). The unmetabolized compound was also present in the urine; following ingestion of 10 mg *N*-phenyl-2-naphthylamine up to 163 μg (average 20 μg in 19 volunteers) was excreted in the urine unchanged in 24 h (Kummer & Tordoir, 1975), indicating that as with 1- and 2-naphthylamine only small quantities of the administered dose appear unchanged in the urine.

Although metabolic studies with chlornaphazine (63) have not been reported, it has been proposed that its metabolism would be similar to that of other nitrogen mustards and would result in the formation of 2-naphthylamine (Cohen et al., 1982).

6.6.2 Short-term tests

Table 16 summarizes data on mutagenicity and other short-term assays for genotoxicity of naphthylamines. The criteria for inclusion under a given type of test are outlined in Section 6.3.2

6.6.3 Carcinogenicity in experimental animals

IARC has determined that there is sufficient evidence for carcinogenicity in experimental animals for 2-naphthylamine (58) and limited evidence for chlornaphazine (63) and 1,5-naphthalenediamine (61) (IARC, 1974, 1982a). 1-Naphthylamine has failed to induce tumours in most evaluated studies (IARC, 1982c).

6.6.4 Effects in humans

6.6.4.1 Systemic Acute poisoning by 2-naphthylamine (58) may result in methaemoglobinaemia and acute haemorrhagic cystitis (ACGIH, 1980). Urinary

Table 16 Short-term tests: naphthylamines

Compound	Compound No.	Positive results				Negative results			
		DNA damage	Mutagenicity	Chromosome anomalies	Other	DNA damage	Mutagenicity	Chromosome anomalies	Other
1-naphthylamine[a]	(57)	X	X	X			X	X	$X_{CT\,SA}$
2-naphthylamine[a,b]	(58)	X	X	X	X_{CT}		X		X_{SA}
N-phenyl-2-naphthylamine[a]	(60)		X				X		X_{CT}
1,5-naphthalenediamine[c]	(61)		X						
chlornaphazine[d,e]	(63)		X	X					

From:
[a] IARC (1982c)
[b] Rosenkranz & Poirier (1979)
[c] IARC (1982a)
[d] Fahmy & Fahmy (1970)
[e] Benedict et al. (1977)

Key:
CT = cell transformation
SA = sperm abnormality

symptoms including cystitis, haematuria and dysuria, and abnormal urinary cytology have also been reported in some cases, prior to diagnosis of bladder tumours, following the treatment of Hodgkin's disease and polycythaemia with chlornaphazine (63), following total doses as low as 2 g (Laursen, 1970; IARC, 1974). Concomitant medication, including cyclophosphamide which is known to induce haemorrhagic cystitis, was used in some cases (Laursen, 1970).

6.6.4.2 Dermatitis Naphthylamines, including notably *N*-phenyl-1-naphthylamine (59) and *N*-phenyl-2-naphthylamine (60) have been shown to be sensitizers (Bandmann and Dohn, 1967). Contact dermatitis resulting from sensitivity to naphthylamines does not, however, seem to be frequent. There are only a few references concerning sensitization to naphthyl compounds. From Amsterdam, van Ketel (1983) has reported a very low incidence of possible sensitization to naphthyl mix (*N*-phenyl-2-naphthylamine and di-(2-naphthyl)-*p*-phenylenediamine). Bieber & Foussereau (1968) described 6 patients with sensitization to *N*-phenyl-2-naphthylamine (Table 9). Boman et al. (1980) described a patient with allergy to *N*-phenyl-2-naphthylamine. This allergen was found to be a grade V allergen (i.e. sensitization rate of 81-100%) on performing the Guinea Pig Maximization test. Naphthyl mix is used in the standard patch tests in different countries. Cronin (1980) reported that in St John's Hospital in London during 1971–76 only 15 of the 4,643 men and 6 of 5,349 women reacted to this mix. The reactions to *N*-phenyl-2-naphthylamine were positive in 7/11 patients with positive reactions to the mix while all 9 tested patients reacted to di-(2-naphthyl)-p-phenylenediamine.

Acne and hypersensitivity to sunlight have been associated with prolonged periods of occupational exposure to *N*-phenyl-2-naphthylamine. Leucoplakia was also reported in the same group (Sielicka-Zuber, 1961).

6.6.4.3 Carcinogenicity There have been several reports of cases of urinary bladder tumours attributed to commercial 1-naphthylamine (57) (IARC, 1974, 1982c), and epidemiological studies considering exposure, either to the compound alone (Table 12), or with concomitant exposures to other aromatic amines (Case et al., 1954; Goldwater et al., 1965; Rubino et al., 1979), have also recorded significant elevations in bladder cancer incidence. Commercial 1-naphthylamine, at the time of the study by Case et al. (1954) on exposure to the compound alone, however, was contaminated with 4-10% 2-naphthylamine. Commercial 1-naphthylamine containing 4–10% 2-naphthylamine was therefore assessed by IARC as being "strongly associated with bladder cancer" in humans (IARC, 1974). More recently, the 2-naphthylamine content of 1-naphthylamine has been significantly reduced to a maximum of 0.5% (IARC, 1974).

Several case reports have linked exposure to 2-naphthylamine with urinary bladder cancer (IARC, 1974). Many epidemiological studies have also demonstrated clearly the association between 2-naphthylamine and bladder cancer in man, either from exposure to the compound alone or exposure to chemicals contaminated by 2-naphthylamine (Table 12), or in conjunction with exposure to other aromatic amines (Case et al., 1954; Goldwater et al., 1965; Mancuso & El-Attar, 1967; Rubino et al., 1979; Morinaga et al., 1982). IARC (1974) has evaluated 2-naphthylamine "either alone or when present as an impurity in other compounds" as being "strongly associated with the occurrence of bladder cancer", and has concluded that "there is no doubt that 2-naphthylamine is a human bladder carcinogen". In addition to

an association with bladder cancer, exposure to 2-naphthylamine and benzidine (45) in the same plant has been implicated in the appearance of pancreatic cancers and second primary cancers at several sites (Section 6.4.4.3).

There is at present no direct evidence associating *N*-phenyl-2-naphthylamine with cancer in humans despite the fact that it may be contaminated with 2-naphthylamine and is known to be metabolized by humans to 2-naphthylamine. However, the limited epidemiological studies of people known to be exposed to *N*-phenyl-2-naphthylamine, or employees in the rubber industry where the compound may have been used, do not permit an assessment of its carcinogenic risk to humans, (IARC, 1978).

Case reports and epidemiological studies have demonstrated an association between the clinical use of chlornaphazine and bladder tumour formation (Table 12). Tumour incidence has been associated with as little as 4 g of the drug. In one study, 9/10 patients who developed bladder tumours were also treated with ^{32}P as sodium phosphate injections. However, no cases of bladder tumours were diagnosed among patients treated with [^{32}P]-sodium phosphate alone. IARC has therefore concluded that co-administration of [^{32}P]-sodium phosphate and chlornaphazine may be carcinogenic in humans (IARC, 1974).

6.7 Aminoazobenzenes

6.7.1 Pharmacokinetics and metabolism

6.7.1.1 Animals As a general rule when considering the more complex azo dyes, highly sulphonated azo dyes, being highly polar compounds, are poorly absorbed from the gut lumen, whereas lipid soluble dyes would be expected to be more readily absorbed. Reduction of polar dyes by gut microflora, following oral dosing, however, may give rise to metabolites which are more readily absorbed.

Early experiments by the Millers demonstrated the binding of *p*-dimethylaminoazobenzene (67) to liver proteins, and similar results were obtained for the related 3'-methyl derivative (69) (Arcos & Argus, 1968). *p*-Aminoazobenzene (65) and *p*-dimethylaminoazobenzene, in common with other azobenzenes, both have high octanol/water partition coefficients (log P = 3.5 and 4.58 respectively) indicating that they are highly lipophilic compounds with therefore a high tendency to bioaccumulate. As is frequently found with highly lipophilic compounds, however, they might be expected to be extensively metabolized (Radding et al., 1977).

Miller & Miller (1983) have recently reviewed the early studies on aminoazo dye metabolism. Azo reduction may occur. The single ring products from azo reduction of *p*-dimethylaminoazobenzene; *N*-acetyl-*p*-aminophenol and *N,N'*-diacetyl-*p*-phenylenediamine have been isolated in the urine of rats administered the dye. Fifty to sixty per cent of administered dye is accounted for by these 2 metabolites (Arcos & Argus, 1974). However, this is not necessarily characteristic of azo dyes and, with other dyes, urinary excretion of unreduced dye and metabolites containing the azo link have been observed (Walker, 1970). The metabolic pathway followed by azo compounds is dependent in part on the mode of administration, the degree of absorption from the gut, especially after oral dosing, and the extent of biliary excretion, particularly after administration by other than oral routes. For example, several studies have demonstrated that i.v. or i.p. administration of azo dye has resulted in urinary or biliary excretion of unreduced dye or metabolites, whereas oral administration has resulted in azo reduction and the excretion of cleavage

products. When azo dyes are administered i.p. or i.v. they may be excreted in the bile and therefore subsequently reach the gut where they are subject to the action of gut flora and possible reabsorption. For example, conjugates of *p*-dimethylaminoazobenzene were observed to be excreted in the bile of rats whereas only single ring metabolites were excreted in the urine (Walker, 1970).

6.7.1.2 Humans Few reports on the pharmacokinetics and metabolism of aminoazobenzenes have been found. 2,4-Diaminoazobenzene (66) found as an impurity in the benzidine-based dye Direct Black 38 has been detected in the urine of workers exposed to the dye (NIOSH, 1980).

6.7.2 Short-term tests

Table 17 summarizes data on mutagenicity and other short-term assays for genotoxicity of aminoazobenzenes. The criteria for inclusion under a given type of test are outlined in Section 6.3.2.

6.7.3 Carcinogenicity in experimental animals

IARC has determined that there is sufficient evidence for carcinogenicity in experimental animals for *o*-aminoazotoluene (70) and *p*-dimethylaminoazobenzene (67) (IARC, 1975). Inadequate evidence of carcinogenicity existed for 2,4-diaminoazobenzene (66) when last evaluated (IARC, 1975).

In a recent prioritization study of around 400 azo dyes for carcinogen bioassay by the NCI, three aminoazo dyes were assigned a high priority: Direct Red 80, Basic Red 18 and Disperse Brown 1 (Helmes et al., 1984).

6.7.4 Effects in humans

6.7.4.1 Systemic There have been occasional reports of acute toxicity due to the cathartic action of some azo food colours, but apart from these there is no direct evidence that the use of such food colours has had an adverse effect on the general population (Radomski, 1974).

6.7.4.2 Dermatitis The sensitizing dyes, present in both men's and women's clothes practically all belong to the Disperse group which was developed for use on synthetic fibres. Chemically many of the Disperse dyes are aminoazobenzenes or derivatives of aminoazobenzenes. The majority of the sensitizers are azodyes. The propensity of Disperse dyes to sensitize is probably related to the difficulty of making them fast on a synthetic fibre, whereas natural fibres hold dyes more readily.

Although, as mentioned, azodyes are weak sensitizers, several of them have been reported to cause contact dermatitis. *o*-Aminoazotoluene (70) used in coloured inks and diacetylaminoazotoluene (71) have been reported to cause allergic reactions including eczema of the hands and arms (Meara & Martin-Scott, 1953; Zina & Bonu, 1965; Castellain, 1967). *p*-Dimethylaminoazobenzene (67) has caused contact dermatitis following handling by factory workers (IARC, 1975) and a dermatitis from an azodye, Acid Yellow 36, used in industrial leather protective shoes has been recorded (Ancona et al., 1982).

Table 17 Short-term tests: aminoazobenzenes

Compound	Compound No.	Positive results				Negative results			
		DNA damage	Mutagenicity	Chromosome anomalies	Other	DNA damage	Mutagenicity	Chromosome anomalies	Other
p-aminoazobenzene[a,b,c]	(65)	X	X		X_{CT}	X	X		X_{CT}
2,4-diaminoazobenzene[c]	(66)**		X						
Bismark Brown G[c]			X					X	
p-dimethylaminoazobenzene[a,c,d,e]	(67)*	X	X		X_{CT}	X	X		X_{DL}
3,5-dimethyl-4-aminoazobenzene[c]									X_{CT}
3,5-dimethyl-4-dimethylaminoazobenzene[c]									X_{CT}
p-methylaminoazobenzene[b,f]	(68)	X	X				X		
4-dimethylamino-3'-methylazobenzene[c]	(69)	X	X		X_{CT}	X			X_{CT}
Methyl Red[c]			X				X	X	
Methyl Orange[c]			X		X_{CT}	X	X		
o-aminoazotoluene[a,b,c]	(70)	X	X			X	X		
Direct Black 17[c]			X						
Acid Yellow 36[c]				X			X		
Basic Red 18[c]			X						
Fast Yellow AB[c]						X	X		

Footnote: Structures of above compounds not listed in Figure 2 can be found in Combes & Haveland-Smith (1982)

From:

[a] Rosenkranz & Poirier (1979)
[b] IARC (1975)
[c] Combes & Haveland-Smith (1982)
[d] Martin et al. (1978)
[e] Weisburger et al. (1978)
[f] Lin & Fok (1973)

Key:

CT = cell transformation
DL = dominant lethal
* commonly known as Butter-Yellow
** commonly known as Chrysoidine or Basic Orange 2

Fuel sensitization may also be due to dyes, among others the yellow dye *p*-dimethylaminoazobenzene which is the most potent sensitizer in fuels (Lamb & Lain, 1951).

6.7.4.3 Carcinogenicity There is very little information on the potential of any aminoazobenzenes to induce cancer in humans, although OSHA does recognize *p*-dimethylaminoazobenzene (67) as a carcinogen (Beard & Noe, 1981). *o*-Aminoazotoluene (70) is an intermediate formed during the manufacture of safranine-T which has been associated with an excess of urinary bladder cancer (Rubino et al., 1979, 1982) (see Section 6.3.4.3 for discussion). A possible connection between the use of azo dyes as food additives and the incidence of intestinal cancers in industrialized countries has been proposed (Chung, 1983). More recently attention has been drawn to the possible association between the use of chrysoidine (2,4-diaminoazobenzene) (66) to dye fishing baits and the incidence of cancer in anglers (Sole, 1984). Bismark Brown, a derivative of chrysoidine, has also been used for this purpose (Cartwright, 1983).

6.8 Toxicity of chemicals used to replace known carcinogenic aromatic amines

Although some chemicals may have been withdrawn from industrial use because of proven carcinogenicity to humans (for example, 2-naphthylamine (58)), it is possible that some of the compounds introduced in their place may also have significant human toxicity. *N*-phenyl-2-naphthylamine (60), for example, was introduced as a rubber antioxidant to replace materials containing free naphthylamine isomers. Not only may *N*-phenyl-2-naphthylamine be contaminated with 2-naphthylamine, it is also metabolized by humans to 2-naphthylamine (Kummer & Tordoir, 1975; NIOSH, 1976). Also several epidemiological studies of dye users suggest that there may be an excess risk of bladder cancer in groups of workers exposed to certain benzidine-based dyes (for example, shoe and leather workers, tailors, textile workers and hairdressers) (Table 4). Since then, a number of reports have considered or referred to the use of substitutes to replace such dyes (NIOSH, 1980). A number of substitutes, however, are based on benzidine-congeners (Table 1) considered to be less toxic than benzidine (45). Available information, however, on the metabolism and carcinogenic effects of most dyes containing these congeners is extremely limited.

It is therefore important to ensure adequate assessment of the toxicity of chemicals proposed for use in place of known toxic materials, and the toxicity of metabolites should also be considered.

Summary

In general, exposure to aromatic amines may be via a variety of routes but the dermal route is considered to be particularly important in humans.

In animal experiments, aromatic amines appear to be distributed mainly to the excretory organs: the liver, gastro-intestinal tract, kidney and bladder. They appear for the most part to be rapidly and extensively metabolized. In many instances only small amounts of the parent amine relative to metabolites are excreted. *N*-Oxidation

and conjugation to form glucuronide-, sulphate- or acetyl-conjugates is common. Metabolism to potentially more toxic compounds may be important, for example, benzidine-based dyes may be reduced to benzidine (45) and *N*-substituted 2-naphthylamines may be dealkylated to 2-naphthylamine (58).

Some aromatic amines have been reported to cause methaemoglobinaemia in experimental animals or humans. A number have also been reported to cause irritation or lesions of the urinary bladder.

Many aromatic amines and related compounds are known to cause allergic contact dermatitis and other sensitization reactions. Allergic contact dermatitis appears to be a significant health effect caused by phenylenediamines and has also been observed with some benzidines, 4,4′-diaminodiphenylmethanes, naphthylamines and aminoazobenzenes. Although patch-testing may assist in the identification of the causative agent, mixed exposures to a variety of chemicals and the possibility of cross-sensitization make it difficult to assess the extent of the problem for most compounds.

A number of aromatic amines are currently considered to be either known or possible human carcinogens. The International Agency for Research on Cancer (IARC) has evaluated several aromatic amines (volumes 1–39 of the Monograph Series) and classified them according to carcinogenic risk. The compounds and processes listed below are categorized according to the latest evaluation.

Known to be carcinogenic to humans
Analgesic mixtures containing phenacetin (19)[a]
Benzidine (45)
2-Naphthylamine (58)
Chlornaphazine (63)
4-Aminobiphenyl (72)
Auramine manufacture (74)[a]

Probably carcinogenic to humans (higher degree of evidence)
o-Toluidine (2)
Phenacetin (19)
Magenta manufacture (75)[a,b]

Probably carcinogenic to humans (lower degree of evidence)
3,3′-Dimethoxybenzidine (48)
3,3′-Dichlorobenzidine (50)
Auramine (74)[c]
Direct Black 38 (76)[c]
Direct Blue 6 (77)[c]
Direct Brown 95 (78)[c]

Several other aromatic amines have been evaluated but there has been insufficient evidence to assess possible carcinogenicity to humans.

At present no attempt can be made to interpret animal data directly in terms of human risk since no objective criteria are available to do so. However, IARC have suggested that for practical purposes it is reasonable to regard the following compounds as if they presented a carcinogenic risk to humans, as sufficient evidence exists for carcinogenicity in animal bioassays:
o-Anisidine (12)
p-Cresidine (15)

p-Chloro-*o*-toluidine (30)
2,4-Diaminotoluene (34)
2,4-Diaminoanisole (36)
4-Chloro-*o*-phenylenediamine (42)
N,N'-Diacetylbenzidine
3,3'-Dimethylbenzidine (46)
4,4'-Methylenedianiline (53)
4,4'-Methylenebis(2-chloroaniline) (54)
4,4'-Methylenebis(2-methylaniline) (55)
p-Dimethylaminoazobenzene (67)
o-Aminoazotoluene (70)
Direct Blue 14 (80)[d]

In humans, aromatic amines, notably benzidines and naphthylamines, have been associated with urinary bladder cancers. Several occupations have been identified in which there would appear to be an unusually high incidence of bladder cancer and also cancers at other sites, either or both of which may be related to exposure to aromatic amines. Such occupations include the chemical and dye industries, rubber and plastics manufacture, and hairdressers and beauticians.

In animal bioassays, aromatic amines cause tumours predominantly of the urinary bladder and liver, although other sites may be involved.

Compounds that have attracted more recent attention with respect to potential human carcinogenicity include 4,4'-methylenebis(2-chloroaniline) (54) and 4,4'-methylenebis (2-methylaniline) (55), last reviewed by IARC in 1974, and 2,4-diaminoazobenzene (66) last reviewed by IARC in 1975. There is also limited epidemiological evidence of an association between increased cancer incidence and occupational exposure to some phenylenediamines used in hair dyes (IARC, 1982a)

For information regarding current studies in cancer epidemiology and toxicity/carcinogenicity testing of aromatic amines, the reader is referred to the latest volumes of the following publications:
Directory of on-going research in cancer epidemiology (IARC); *Information bulletin on the survey of chemicals being tested for carcinogenicity* (IARC); and *Computerized listing of chemicals being tested for toxic effects (CCTTE)* (IPCS/IRPTC).

[a]The compound(s) responsible for the carcinogenic effect in humans cannot be specified.
[b]See footnote on page 76
[c]Technical grade
[d]Commercial grade

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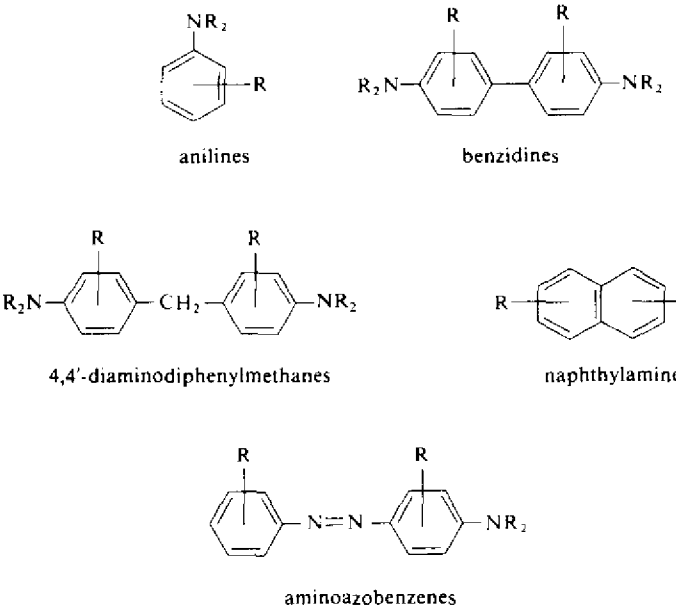
APPENDIX

Figure 1 Parent structures of the different groups of aromatic amines considered in this document



Key: R = either a hydrogen atom or one or more ring substituents (substituents need not be the same)

R_2 = either 2 hydrogen atoms (primary amine) or 1 hydrogen atom plus an alkyl or aryl substituent (secondary amine) or a combination of 2 such substituents (tertiary amine)

Note: For benzidine dyes and pigments and some aminoazobenzene derivatives, R_2 = an azo-linked compound

Figure 2 Key to structures of aromatic amines discussed in text

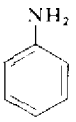
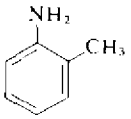
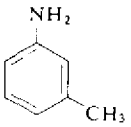
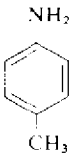
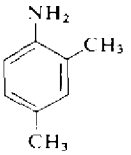
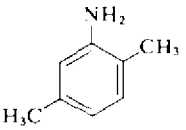
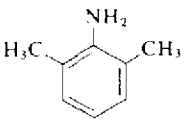
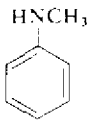
Compound number	Name used in text	Chemical abstract name	Structure
Anilines			
(1)	aniline	benzenamine	
(2)	*o*-toluidine	2-methylbenzenamine	
(3)	*m*-toluidine	3-methylbenzenamine	
(4)	*p*-toluidine	4-methylbenzenamine	
(5)	2,4-xylidine	2,4-dimethylbenzenamine	
(6)	2,5-xylidine	2,5-dimethylbenzenamine	
(7)	2,6-xylidine	2,6-dimethylbenzenamine	
(8)	*N*-methylaniline	*N*-methylbenzenamine	

Figure 2 contd.

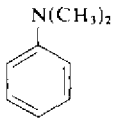
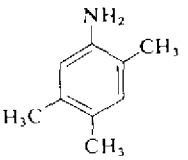
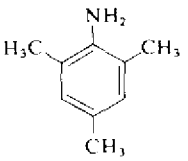
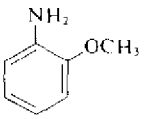
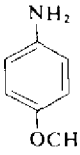
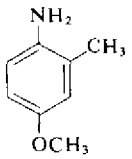
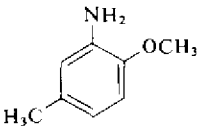
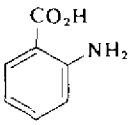
Compound number	Name used in text	Chemical abstract name	Structure
(9)	*N,N*-dimethylaniline	*N,N*-dimethylbenzenamine	
(10)	2,4,5-trimethylaniline	2,4,5-trimethylbenzenamine	
(11)	2,4,6-trimethylaniline	2,4,6-trimethylbenzenamine	
(12)	*o*-anisidine	2-methoxybenzenamine	
(13)	*p*-anisidine	4-methoxybenzenamine	
(14)	*m*-cresidine	4-methoxy-2-methylbenzenamine	
(15)	*p*-cresidine	2-methoxy-5-methylbenzenamine	
(16)	anthranilic acid	2-aminobenzoic acid	

Figure 2 contd.

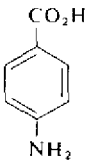
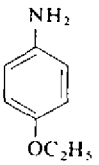
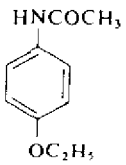
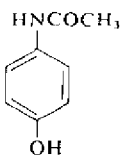
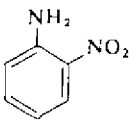
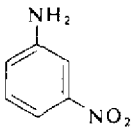
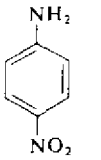
Compound number	Name used in text	Chemical abstract name	Structure
(17)	*p*-aminobenzoic acid	4-aminobenzoic acid	
(18)	phenetidine	4-ethoxybenzenamine	
(19)	phenacetin	*N*-(4-ethoxyphenyl)acetamide	
(20)	paracetamol (acetaminophen)	*N*-(4-hydroxyphenyl)acetamide	
(21)	*o*-nitroaniline	2-nitrobenzenamine	
(22)	*m*-nitroaniline	3-nitrobenzenamine	
(23)	*p*-nitroaniline	4-nitrobenzenamine	

Figure 2 contd.

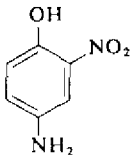
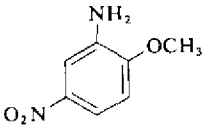
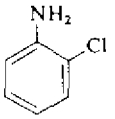
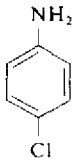
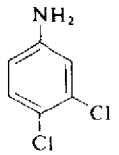
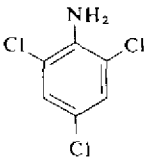
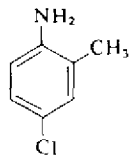
Compound number	Name used in text	Chemical abstract name	Structure
(24)	4-amino-2-nitrophenol	4-amino-2-nitrophenol	
(25)	5-nitro-*o*-anisidine	2-methoxy-5-nitrobenzenamine	
(26)	2-chloroaniline	2-chlorobenzenamine	
(27)	4-chloroaniline	4-chlorobenzenamine	
(28)	3,4-dichloroaniline	3,4-dichlorobenzenamine	
(29)	2,4,6-trichloroaniline	2,4,6-trichlorobenzenamire	
(30)	*p*-chloro-*o*-toluidine	4-chloro-2-methylbenzenamine	

Figure 2 contd.

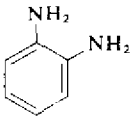
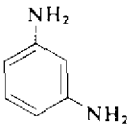
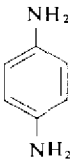
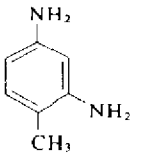
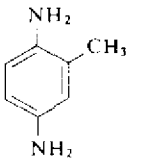
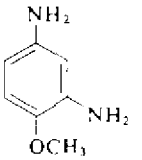
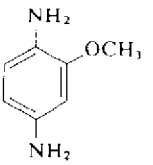
Compound number	Name used in text	Chemical abstract name	Structure
Phenylenediamines			
(31)	*o*-phenylenediamine	1,2-benzenediamine	
(32)	*m*-phenylenediamine	1,3-benzenediamine	
(33)	*p*-phenylenediamine	1,4-benzenediamine	
(34)	2,4-diaminotoluene	4-methyl-1,3-benzenediamine	
(35)	2,5-diaminotoluene	2-methyl-1,4-benzenediamine	
(36)	2,4-diaminoanisole	4-methoxy-1,3-benzenediamine	
(37)	2,5-diaminoanisole	2-methoxy-1,4-benzenediamine	

Figure 2 contd.

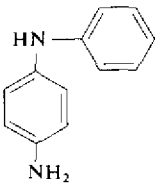
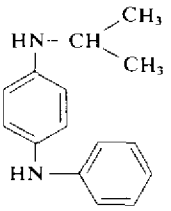
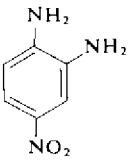
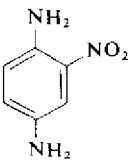
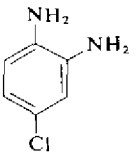
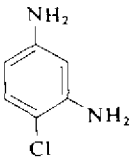
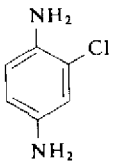
Compound number	Name used in text	Chemical abstract name	Structure
(38)	*N*-phenyl-*p*-phenylenediamine	*N*-phenyl-1,4-benezenediamine	
(39)	*N*-*i*-propyl-*N'*-phenyl-*p*-phenylenediamine	*N*-(1-methylethyl)-*N'*-phenyl-1,4-benzenediamine	
(40)	1,2-diamino-4-nitrobenzene	4-nitro-1,2-benzenediamine	
(41)	1,4-diamino-2-nitrobenzene	2-nitro-1,4-benzenediamine	
(42)	4-chloro-*o*-phenylene-diamine	4-chloro-1,2-benzenediamine	
(43)	4-chloro-*m*-phenylene-diamine	4-chloro-1,3-benzenediamine	
(44)	2-chloro-*p*-phenylene-diamine	2-chloro-1,4-benzenediamine	

Figure 2 contd.

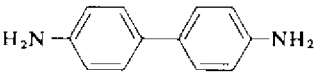
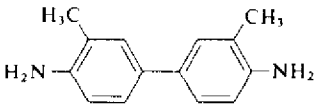
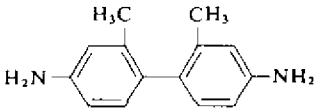
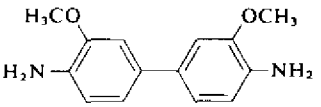
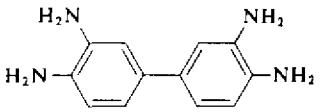
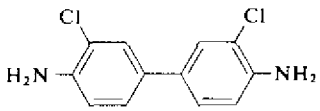
Compound number	Name used in text	Chemical abstract name	Structure
Benzidines			
(45)	benzidine	[1,1'-biphenyl]-4,4'-diamine	
(46)	3,3'-dimethyl-benzidine	3,3'-dimethyl-[1,1'-biphenyl]-4,4'-diamine	
(47)	2,2'-dimethyl-benzidine	2,2'-dimethyl-[1,1'-biphenyl]-4,4'-diamine	
(48)	3,3'-dimethoxy-benzidine	3,3'-dimethoxy-[1,1'-biphenyl]-4,4'-diamine	
(49)	3,3'-diamino-benzidine	[1,1'-biphenyl]-3,3'-4,4'-tetramine	
(50)	3,3'-dichloro-benzidine	3,3'-dichloro-[1,1'-biphenyl]-4,4'-diamine	

Figure 2 contd.

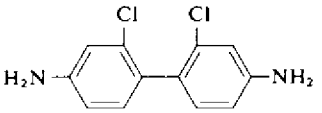
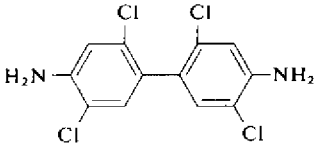
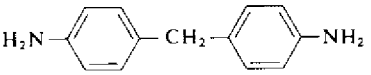
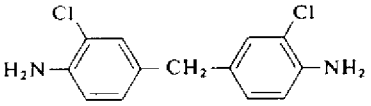
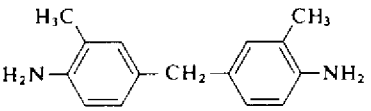
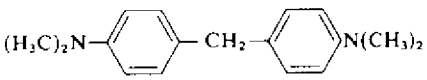
Compound number	Name used in text	Chemical abstract name	Structure
(51)	2,2'-dichloro-benzidine	2,2'-dichloro-[1,1'-biphenyl]-4,4'-diamine	
(52)	2,2',5,5'-tetrachloro-benzidine	2,2',5,5'-tetrachloro-[1,1'-biphenyl]-4,4'-diamine	
4,4'-Diaminodiphenylmethanes			
(53)	4,4'-methylene-dianiline	4,4'-methylenebis-benzenamine	
(54)	4,4'-methylenebis-(2-chloroaniline)	4,4'-methylenebis-[2-chlorobenzenamine]	
(55)	4,4'-methylenebis-(2-methylaniline)	4,4'-methylenebis-[2-methylbenzenamine]	
(56)	4,4'-methylenebis-(*N*,*N*-dimethyl-aniline)	4,4'-methylenebis-[*N*,*N*-dimethylbenzenamine]	

Figure 2 contd.

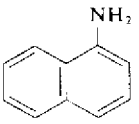
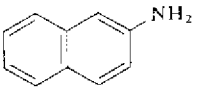
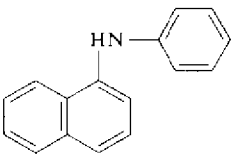
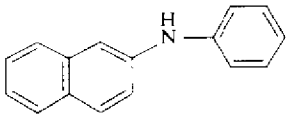
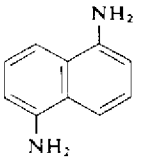
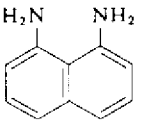
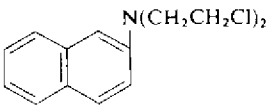
Compound number	Name used in text	Chemical abstract name	Structure
Naphthylamines			
(57)	1-naphthylamine	1-naphthalenamine	
(58)	2-naphthylamine	2-naphthalenamine	
(59)	*N*-phenyl-1-naphthylamine	*N*-phenyl-1-naphthalenamine	
(60)	*N*-phenyl-2-naphthylamine	*N*-phenyl-2-naphthalenamine	
(61)	1,5-naphthalenediamine	1,5-naphthalenediamine	
(62)	1,8-naphthalenediamine	1,8-naphthalenediamine	
(63)	chlornaphazine	*N*,*N*-bis(2-chloroethyl)-2-naphthalenamine	

Figure 2 contd.

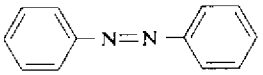
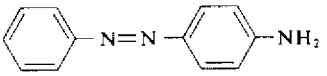
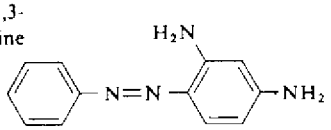
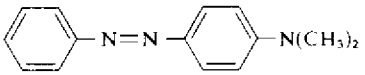
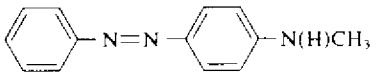
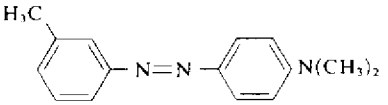
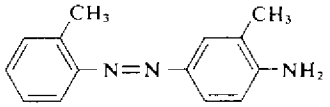
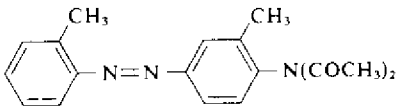
Compound number	Name used in text	Chemical abstract name	Structure
Aminoazobenzenes			
(64)	azobenzene	diphenyldiazine	
(65)	*p*-aminoazobenzene	4-(phenylazo)benzenamine	
(66)	2,4-diaminoazo-benzene	4-(phenylazo)-1,3-benzenediamine	
(67)	*p*-dimethylaminoazo-benzene	*N,N*-dimethyl-4-(phenylazo)-benzenamine	
(68)	*p*-methylaminoazo-benzene	*N*-methyl-4-(phenylazo)-benzenamine	
(69)	4-dimethylamino-3'-methylazobenzene	*N,N*-dimethyl-4-[(3-methylphenyl)azo]-benzenamine	
(70)	*o*-aminoazotoluene	2-methyl-4-[(2-methyl-phenyl)azo]benzenamine	
(71)	diacetylaminoazo-toluene	*N*-acetyl-*N*(2-methyl-4-[(2-methylphenyl)azo]-phenyl)acetamide	

Figure 2 contd.

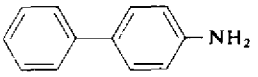
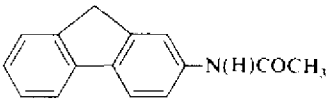
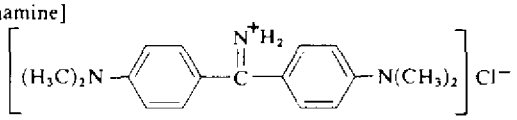
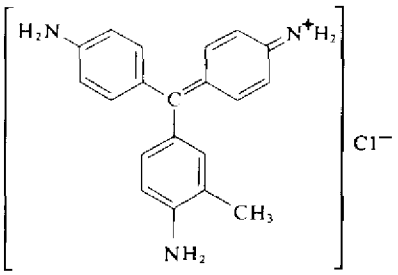
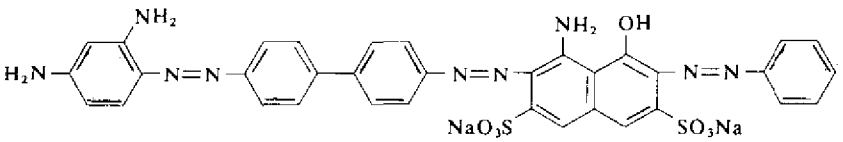
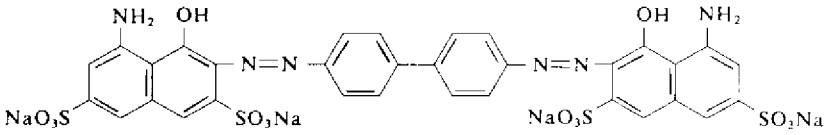
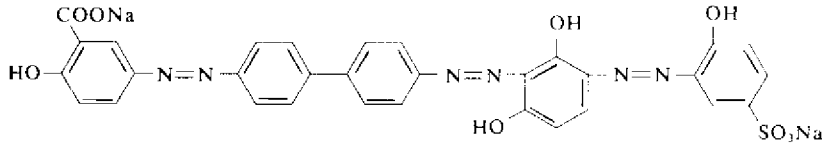
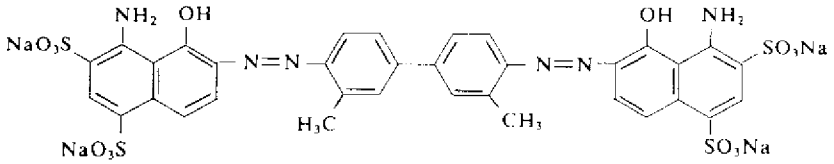
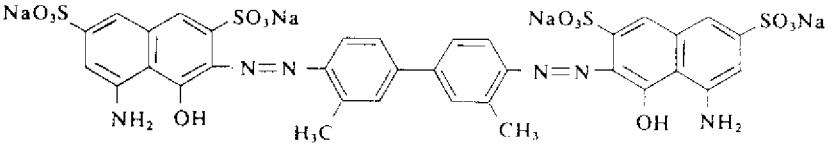
Compound number	Name used in text	Chemical abstract name	Structure
Miscellaneous			
(72)	4-aminobiphenyl	[1,1'-biphenyl]-4-amine	
(73)	2-acetylamino-fluorene	*N*-9*H*-fluoren-2-yl-acetamide	
(74)	auramine	4,4'-carbonimidoyl-bis[*N*,*N*-dimethyl-benzenamine]	
(75)	magenta	4-[(4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)-methyl]-2-methylbenzenamine, monochloride	
(76)	Direct Black 38	2,7-Naphthalenedisulphonic acid, 4-amino-3-[(4'-((2,4-diaminophenyl)azo)-(1,1'-biphenyl)-4-yl)azo]-5-hydroxy-6-(phenylazo)-, disodium salt	

Figure 2 contd.

Compound number	Name used in text	Chemical abstract name	Structure
(77)	Direct Blue 6	2,7-Naphthalenedisulphonic acid, 3,3'-[(1,1'-biphenyl)-4,4'-diylbis(azo)]-bis-(5-amino-4-hydroxy)-, tetrasodium salt	
(78)	Direct Brown 95	Benzoic acid, 5-[(4'-((2,6-dihydroxy-3-((2-hydroxy-5-sulphophenyl)azo)phenyl)azo-(1,1'-biphenyl)-4-yl)azo]-2-hydroxy-, disodium salt	
(79)	Direct Blue 53	1,3-Naphthalenedisulphonic acid, 6,6'-[(3,3'-dimethyl-(1,1'-biphenyl)-4,4'-diylbis-(azo)]-bis(4-amino-5-hydroxy)-, tetrasodium salt	
(80)	Direct Blue 14	2,7-Naphthalenedisulphonic acid, 3,3'-[(3,3'-dimethyl-(1,1'-biphenyl)-4,4'-diylbis-(azo)]-bis(5-amino-4-hydroxy)-, tetrasodium salt	

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The objective of the MARC core research programme is to develop and apply techniques for the assessment of pollution problems of global, regional or local significance. The programme is mainly carried out by means of reviews which synthesize existing relevant knowledge from a wide range of disciplines.

MARC currently receives financial support from the United Nations Environment Programme (UNEP) and the World Health Organization (WHO).

The results of the Centre's work are published mainly in the MARC report series as Technical Reports, General Reports and Research Memoranda, or in professional journals.

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MARC General Reports are synoptic reviews of environmental topics relevant to monitoring and assessment. Each is written by a specialist in the field, keeping a sense of perspective across the whole breadth of the subject. Their main purpose is to be usable by those environmental scientists and managers who are not expert in the topic being covered but who need to obtain a broader, multidisciplinary understanding of monitoring and assessment problems.

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MARC Research Memoranda are short informal reports related to MARC's ongoing work programme. They may include ideas, data and bibliographical material useful to monitoring and assessment. Their main purpose is to act as a forum for wider discussion.

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17 *Analysis of the effects of lead in tissue upon human health using dose-response relationships* by J. K. Piotrowski and B. J. O'Brien

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