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Aromatic amines and cancer

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Epidemiologicalevidence on the relation between aromatic amines and cancer risk is reviewed. In particular, cancer risk in humans resulting from exposure to aromatic amines from occupational sources and tobacco smoking is assessed with reference to ecologic, cohort, and case-control studies. Seven arylamines have been classified by the International Agency for Research on Cancer: benzidine-baseddyes and MOCA (4,4'-methylene bis 2-choloroaniline) were considered 'probably' carcinogenic, Group 2A, because of a high level of evidence in experimental animals; two occupational chemicals (2-naphthylamine and benzidine), one drug (Chlornaphazine), and two manufacturing processes (manufacture of auramine and magenta) were included in Group 1 on the basis of 'sufficient' evidence of carcinogenicity in humans. Occupational exposures to aromatic amines explain up to 25 percent of bladder cancers in some areas of Western countries; these estimates might be higher in limited areas of developing countries. Aromatic amines contaminate the ambient air as a component of environmental tobacco smoke. There is increasing evidence that the excess of bladder cancer in smokers is attributable to aromatic amines rather than to other contaminants of tobacco smoke such as polycyclic aromatic hydrocarbons (PAH). A modulating role in the risk of bladder cancer associated with exposure to aromatic amines is played by metabolic polymorphisms, such as the N-acetyltransferase genotype, raising important social and ethical issues. The consistent observation of a difference between men and women in bladder cancer risk, after allowing for known risk factors, suggests consideration of gender-related biological determinants for future investigation. *Cancer Causes and Control* 1997, 8, 346-355

Key words: Arylamines, bladder cancer, occupational exposure, tobacco.

Introduction

Exposure to aromatic amines (arylamines) occurs in different industrial and agricultural activities. Aromatic amines have been used as antioxidants in the production of rubber and in cutting oils, as intermediates in azo dye manufacturing, and as pesticides. They are a common contaminant in several working environments, including the chemical and mechanic industries and aluminum transformation. Arylamine-based dyes are used widely, particularly in the textile industry. An important problem is the transfer of aromatic amines (including carcinogenic compounds) from developed to developing countries.[1] Arylamines contaminate the ambient air where smokers are present.[2]

The site which has been associated causally with an increased risk of cancer in subjects exposed to aromatic amines is the bladder; we will not systematically review the evidence concerning other sites. The most stringent evidence comes from large cohort investigations conducted in the 1950s in the British chemical industry. We will consider cohort studies in detail, andcase-control and ecologic studies only when clear information on exposure to aromatic amines was available. Carcinogenic arylamines such as 2-naphthylamine have been banned in the United Kingdom since 1967 (Carcinogenic Substances Regulation)[3] and in other Western countries subsequently.[4] The International Labour Office had

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already concluded in 1921, based on early observations in humans, that 2-naphthylamine and benzidine were carcinogenic.'

Ecologic studies

Bladder cancer was found to be increased in ecologic studies of geographic areas contaminated with carcinogens, such as the Drake Superfund site in Pennsylvania (United States), an area where different chemical companies had manufactured or stored over decades carcinogens as 2-naphthylamine and benzidine.'

A study by Dolin' computed standardized mortality ratios (SMR) for bladder and lung cancer in 400 districts in England and Wales (UK) in the years 1969-80 and detected an increase in bladder cancer risk among males for textile related occupations (involving exposure to azo dyes) and among females for engineering trades (involving exposure to aromatic amines in cutting fluids).

A death certificate study conducted among 2,457 males deceased for bladder cancer in Great Britain' in the years 1965-80 used a job-exposure matrix to infer exposure to aromatic amines. The SMRs for aromatic amines were 120 (95 percent confidence interval [CI] = 101-141) for 'some exposure,' 123 (CI = 102-147) for low exposure, and 108 (CI = 70-160) for high exposure.

A French study' of male farmers and farm laborers in 89 geographic units, in the years 1984-86, was based on a pesticide exposure index (PEI), and found a relative risk (RR) for bladder cancer of 1.14 (CI = 1.07-1.22) among the heavily exposed. The authors claim that herbicides used in vineyards include diuron which can be contaminated by aromatic amines.

Cohort studies

Following anecdotal observations in humans and experimental work in dogs," the carcinogenicity of arylamines such as 2-naphthylamine and benzidine was demonstrated clearly in the 1950sin a large epidemiologic investigation of British chemical workers." Case and colleagues were able to obtain from the chemical industry nominal rolls for all workers, including information on job titles and the chemicals they manufactured. Overall, 4,622 men in 21 firms were enrolled, and mortality from bladder cancer was ascertained. The observed (Obs) deaths exceeded by far those expected (Exp) among workers exposed to 2-naphthylamine (Obs/Exp Ratio = 87) and benzidine (O/E = 14), while lower excesses were reported for aniline (O/E = 4.5) and I-naphthylamine (O/E = 9). However, subsequent studies did not confirm that the latter substances were carcinogenic, and the apparent excess was probably due to contamination from 2-naphthylamine."

Other studies have reported considerable increased risks of bladder cancer in workers exposed to 2-naphthylamine, benzidine, and 4-aminobiphenyl;[13-25] the results of such studies are summarized in Table 1. Some of the studies did not have a formal epidemiologic design and/or were based on very small numbers.

In one case, all 15 workers involved in distilling of 2-naphthylamine in a small plant in England developed bladder cancer.''This observation is important, because it shows that in exceptional situations (high levels of exposure to potent carcinogens), individual susceptibility is irrelevant. An interesting observation that has been made both in the UK and in the US is that after banning of carcinogenic arylamines, the incidence of bladder cancer in the chemical industry has decreased considerably.[12,21]

Other arylamines, in addition to those in Table 1, have been shown to be carcinogenic to humans. Chlornaphazine, a chloroethyl-derivative of 2-naphthylamine, has been used as a drug for the treatment of polycythemia vera. Among 61 patients treated in 1954-62, 13 developed cancer of the bladder and eight had abnormal urinary cytology;'*this chemical is included in Group 1 (human carcinogens) according to the International Agency for Research on Cancer (IARC)."

0-toluidine has also been suggested as a bladder carcinogen. In their study, Rubino *et al*[16] found a 62-fold increase in bladder cancer risk in workers exposed jointly to o-toluidine and 4,4'-methylene bis(2-methylaniline) both carcinogenic to experimental animals." The excess was based on five cases. Stasik[26] reported a 72-fold increase (eight cases) among workers exposed both to o-toluidine and to 4-chloro-o-toluidine. More recently, a study conducted by the US National Institute for Occupational Safety and Health: concerning a cohort of workers exposed jointly to o-toluidine and aniline, found high RRs (up to 27 for those exposed for 10 or more years). None of these investigations enables us to evaluate separately the contribution of o-toluidine to the excess of bladder cancer. 0-toluidine is clearly carcinogenic in experimental animals, whereas the data are less convincing for aniline." In Germany, in a group of 49 workers exposed to 4-chloro-o-toluidine in the synthesis of chlordimeform, seven cases of bladder cancer occurred, a number about 50 times higher than expected."

Also, two industrial processes (manufacturing of auramine and magenta) have been included in Group 1 (human carcinogens) by IARC Working Groups, although the single responsible carcinogens are not known. In the case of magenta, ortho-toluidine and 4,4'-methylene bis (2-methylaniline) have been suspected."

In addition to bladder cancer, esophageal tumors have been found in excess in two of the studies shown in Table 1: Rubino *et al*,[16] in a plant where workers were exposed to different aromatic amines, reported an O/E ratio of

Table 1. Occupational arylamines with clear evidence of carcinogenity to humans; cohort size is based, whenever possible, on subjects exposed to the specific chemical

Study (ref)	Year	No. of observed deaths or cases	OIE [a]	Cohort size (No.)
2-naphthylamine				
Case *et al*[11]	1954	26	87	4,622[b]
Mancuso & El-Attar[13]	1967	18	30	640
Goldwater *et al*[1]	1965	12		48
Schulte *et al*[15]	1985	13	3.9	1,385b
Rubino *et al*[16]	1982	6	150	30
Bulbulyan *et al*[17]	1995	9	18.9	514 men and 87 women
Benzidine				
Case *et ai*[11]	1954	10	14	4,622'
Mancuso & El-Attar[13]	1967	16	30	640
Zavon *et al*[18]	1973	13		25
Tsuchiya *et al*[19]	1975	72		1,015
Rubino *et al*[16]	1982	5	83	65
Horton *et al*[20]	1977	13		5
Meigs *et al*[21]	1986	6	130	105[c]
Bi *et al*[22]	1992	9	45.7	266
Bulbulyan *et al*[17]	1995	17	13.2	514 men and 87 women
4-aminobiphenyl				
Melamed[23]	1972	43		541
Melick *et al*[24]	1971	53		315
Zack & Gaffey[25]	1983	9	10	884

[a] O/E = observedlexpected.
[b] Total cohort, with miscellaneous exposure to arylamines.
[c] Heavy exposure.

4.72 (statistically significant, based on five cases); and Bulbulyan *et al*[17] reported a ratio of **3.48** (five cases in a group exposed to benzidine and 2-naphthylamine). In contrast, the cohort study by Meigs *et al*[21] on US workers exposed to benzidine did not find any excess in addition to bladder cancer.

Case-control studies and other relevant epidemiologic evidence

Many case-control studies have considered job titles and industrial activities potentially involving exposure to aromatic amines;[29-33] only those studies relying on some form of assessment of exposure to aromatic amines have been included in Table **2.** Increased RRs are obvious in investigations by Bonassi *et al*[29] in Italy, Schumacher *et al*[30] in the US, and Steineck *et al*[31] in Sweden; whereas, results are equivocal in the large study by Siemiatycki *et al*[33] in Montreal (Quebec, Canada). The latter observation may be attributed to imperfect knowledge of specific exposures or to the actual absence of carcinogenic arylamines among the industrial activities surveyed.

One of the major limitations of many epidemiologic studies is the lack of detailed information on exposure to individual chemicals. However, a few clues are worth mentioning because of the consistency of the high prevalence of exposure, and the possible contamination of exposure by arylamines. One **is** represented by jobs with exposure to combustion gases and soot from coal; in addition to polycyclic aromatic hydrocarbons, such jobs involve exposure to arylamines (Table 3).[34-39] For example, in a cohort study of coal carbonizing workers, 2-naphthylamine was found in a sample from tar volatiles.[34] A peculiar observation is represented by the high RRs found by Tremblay and colleagues in Canada for exposure to benzopyrene." In fact, contamination from 2-naphthylamine and 4-aminobiphenyl was documented in the same working environments. Another type of exposure with likely contamination by arylamines (*e.g.*, phenyl-l-naphthylamine) is exposure to cutting oils and cutting fluids, where excess risks of bladder cancer have been repeatedly shown.[50]

Occupation as hairdresser or barber is classified in Group 2A (probable carcinogen) by IARC." The pres-

Table 2. Bladder cancer and occupational exposure to aromatic amines (AA): case-control or linkage studies **1989-95**

Study **(ref)** Year	Country	Gender	**Cases/controls**	Results **OR/RR/MOR** (CI or **P** value)
Bonassi *et al*[29] **1989**	Italy	M	**1211342**	High exposure to AA adjusted for PAH exposure: OR = **3.6** (CI = **1.6-.8.1**) High exposure to AA and possible exposure to PAH: OR = **3.8** (CI = **1.99-7.29**) High exposure to AA and definite exposure to PAH: OR = **4.8** (CI = **0.6-36.1**)
Schumacher *et al*[30] **1989**	USA	M + F	**4171877**	Males: Exposure to benzidine and 2-naphthylamine: **21** cases, **38** controls: < **10** years duration: **OR = 1.0** (CI = **0.5-2.1**) ≥ **10** years duration: OR = **1.6** (CI = **0.6-4.1**)
Steineck *et al*[31,b] **1989**	Sweden	M	**10,123** cases	Exposure to dyes: RR = **1.8** (CI = **1.2-2.6**)
Hours *et al*[32] **1994**	France	M + F	**1161232**	Cutting fluids: OR = **2.6** (CI = **1.2-5.4**) Inks: women **OR** = **14.0** (CI = **1.8-106.5**) Pyrolysis and combustion products: ***OR*** = **2.3** (CI = **1.O-4.0**)
Siemiatycki *et al*[33] **1994**	Canada	M	**48411,879**	(i) Probable exposure to aromatic amines: OR = **1.3** (CI = **0.9-2.0**) (ii) Textile dyers and finishers > **10** years of exposure: OR = 10.8 (CI = **1.0-120**)

[a] Abbreviation: RR = relative risk; OR = odds ratio; MOR = mortality **odds** ratio; **CI** = **95 %** confidence intervals; PAH = polycyclic aromatic hydrocarbons.

[b] Swedish Cancer-Environment Study (a linkage study using a job-exposure matrix).

ence of aromatic amines with different degrees of evidence of carcinogenicity is documented in some cosmetic products, such as auramine (2B) in brilliantines, CI Disperse Blue 1 (2B) and HC Blue No. 1 in semipermanent hair dyes used in the past.

The contribution of studies in animals

The first demonstration of an excess **risk** of bladder cancer after exposure to some arylamines came from anecdotal observations in humans and, almost at the same time, from studies in animals. This occurred before formal epidemiologic studies were conducted. Arylamines are one of the best examples of the predictivity of animal experiments for human risk. A recent review has reported that 25 to 30 percent of all agents that have been associated causally with human cancer were identified first as carcinogens in experimental animals.[52] These authors have suggested that a clearer understanding of cancer induction at the molecular level will allow the design of more predictive animal models.

The reference to evidence in experimental animals has been crucial in the classification of some aromatic amines for their carcinogenicity to humans. Benzidine-based dyes and MOCA (4,4'-methylene bis 2-choloroaniline) have been classified by IARC Working Groups as 'probable' carcinogens, and para-chloroaniline and ortho-toluidine as 'possible' carcinogens, on the basis of strong evidence in animals. More specifically, the evaluation for MOCA was justified (even in the absence of direct evidence of carcinogenicity in humans) by sufficient evidence of carcinogenicity in animals and by mechanistic data showing that the same DNA adduct (a deoxyadenosin-8-yl derivative) can be found in rats and in heavily exposed workers.[51] Three benzidine-based dyes (Direct Black **38,** Direct Blue ***6,*** and Direct Brown 95) were considered as 'probably' carcinogenic to humans because their metabolism in humans leads to the formation of free benzidine," and on the basis of 'sufficient' evidence of carcinogenicity in animals.

Studies in animals (rats and mice) have shown excesses of hepatocellular carcinomas after exposure to benzidine, benzidine-based dyes, 4-aminobiphenyl, or 2-naphthylamine. The rarity of this cancer in Western populations has implied very low statistical power to detect an excess in epidemiologic studies. In contrast, human studies have shown an excess of esophageal cancers that has not been observed clearly in animals.

Table 3. Combustion gases/soot from coal, exposure to policyclic aromatic hydrocarbons: relative risk (RR) and 95% confidence intervals (CI) for bladder cancer in epidemiologic studies[a]

Study (ref)	Year	Titlelexposure	RR	(CI)
Cohort studies				
Doll *et al*[34]	1972	Coal carbonizers	2.4	(1.1-4.3)
Redmond *et al*[35]	1972	Coke oven workers	1.2	(0.1-4.2)
Hammond *et al*[36]	1976	Roofers and waterproofers		
		Duration = 9-19 yrs	0.8	(0.1-3.0)
		Duration = 20+ yrs	1.7	(0.9-2.9)
Gustavsson *et al*[37]	1988	Chimney sweeps	2.3	(1.4-3.4)
Steineck *et al*[38]	1988	Combustion gases from coal	1.2	(1.o-1.4)
Case-referent studies				
Population-based				
Howe *et al*[39]	1980	Glass processors	6.0	(0.7-276)
McLaughlin *et al*[40]	1983	Coal/natural gas	2.9	(1.0-8.2)
		soot	3.0	(0.9-8.9)
Silverman *et al*[41]	1983	Stationary firemen	1.8	(0.7-5.0)
		Ore refining and foundry occupations	0.5	(0.2-1.4)
		Metal heaters	3.0	(0.3-28)
		Glass manufacture	5.9	(0.7-50)
Mommsen[42]	1984	Blacksmiths	5.0	(0.6-236)
Schoenberg *et al*[43]	1984	Cooks	1.3	(0.8-2.3)
Theriault et al[44]	1984	Documented exposure to benzopyrene		
		Duration = 10-19 yrs	6.8	(2.6-17.8)
		Duration = 20+ yrs	2.4	(3.3-46.1)
Morrison *et al*[45]	1985	England, cooks	1.0	(0.7-1.6)
		Japan, cooks	1.0	(0.4-2.4)
		US, cooks	1.2	(0.7-2.1)
		England, coal and coke	0.8	(0.6-1.3)
		Japan, coal and coke	1.3	(0.6-2.6)
		US, coal and coke	1.1	(0.5-2.0)
Hospital-based				
	1968	Smiths	7.6	(0.4-128)
Tola *et al*[47]	1980	Smiths	0.4	—
		Foundry workers	2.0	—
Vineis and Magnani[48]	1985	Brickyards	2.0	(0.9-4.5)
		Foundries	0.7	(0.4-1.3)
Tremblay *et al*[49 b]	1995	Benzo-a-pyrene (BaP) μg/m^3 years		
		-99.9	1.9	(1.1-3.4)
		-199.9	6.1	(3.1-12.3)
		-299.9	5.6	(2.9-10.4)
		300+	3.8	(1.9-7.8)

[a] Four studies for which the study base was not defined were deleted from the table.

[b] Presence of 2-naphthylamine and 4-aminobiphenyl; 4-aminobiphenyl TWA (time-weighted average) concentration.range in 1990: < 1 to 410 ng/m.^{3} No confounding from smoking. The risk for each year of exposure to BaP at a concentration of 1 μg/m^3 increased by 1.7% (0.8%-3.2%).

The contribution of occupational exposure to bladder cancer (attributable risks)

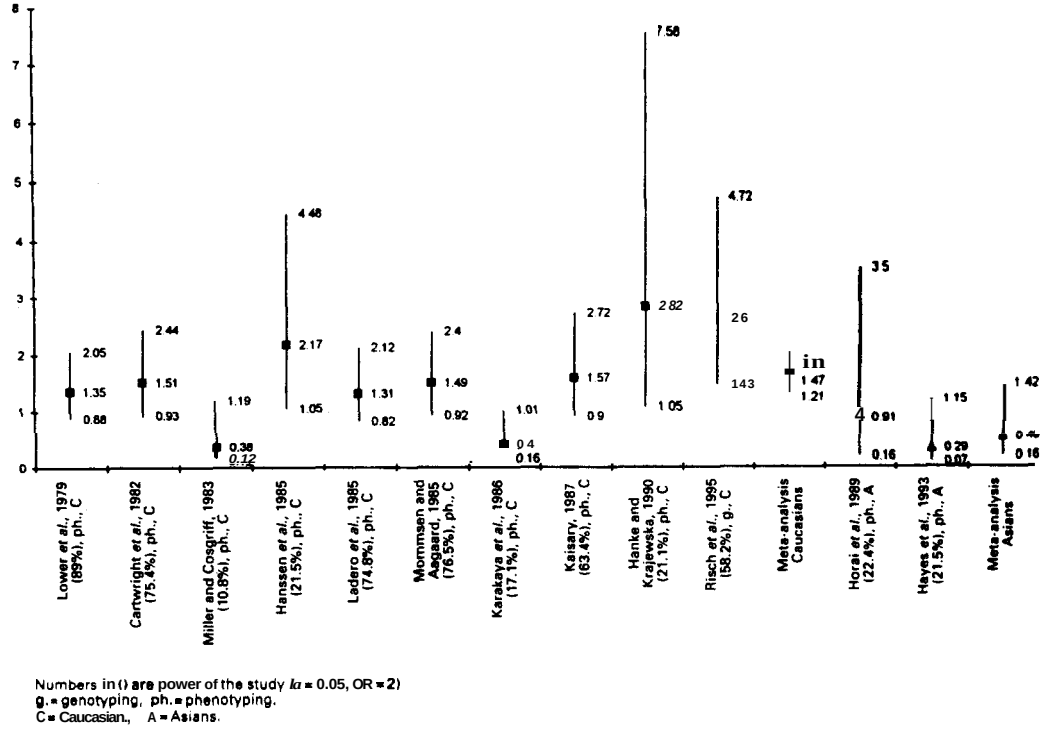
A legitimate question is how many bladder cancers are attributable to occupational exposure to arylamines. The answer is quite uncertain, since well-documented exposure to arylamines has been associated with increased risk of bladder cancer in cohort studies, which do not easily allow the estimation of attributable risks. This is due to the fact that, generally, an estimate of the proportion exposed in the general population (exposure prevalence) is not available from cohort studies. In other investigations, particularly of the case-referenttype, increased risks for job titles, without knowledge on arylamine exposure, were measured.

An attempt to estimate systematically the population attributable risks (PAR) was made by Vineis and Simonato,[53] using evidence from the published case-control studies which adjusted the estimates by smoking habits. In all the PAR estimates, workers of dye-producing plants, rubber workers, and gas workers were included, under the assumption that they were exposed to arylamines. In addition, three criteria to include job titles into the PAR estimates were used. According to the first (the less stringent) criterion, the job titles associated to bladder cancer risk (OR greater than 1.0) in all the published studies, and showing a statistically significant association in at least one study, were included. According to the second criterion, a further condition was that the proportion of exposed controls was at least five percent. According to the most stringent criterion, the same conditions applied but only associations which reached statistical significance in at least two studies were considered (Criterion 111). As a result of this exercise, the PAR estimates varied more ***between*** than *within* studies, suggesting that the criteria used to estimate the PAR are less important than the proportion of workers considered to be exposed in different geographic areas.

These estimates suffer from several limitations: (i) the classification of 'exposed' workers is based on job titles and not on actual knowledge of chemical exposure; (ii) the classification of workers within job categories is affected by errors which tend to entail underestimation of risks; and (iii) different studies have been conducted with different methods and different classification schemes. Nevertheless, the epidemiologic exercise suggests that the proportion of bladder cancers attributable to occupation varies across geographic areas, depending on the prevalence of exposed workers, from a minimum of about zero to a maximum of 20 to 25 percent.

The estimates described refer exclusively to developed countries. An important problem, however, is the bladder cancer burden from arylamine exposure in developing countries. Information is scanty, but it is reasonable to think that such exposures may be widespread due to transfer from developed countries. Tire production involving the use of aromatic amines increased about sevenfold in Brazil and more than 20-fold in India, between **1961** and 1987.[54] India, Mexico, and Brazil are major producers of magenta and para-chloroaniline.[51,55] Epidemiologic studies have documented the relevance of the problem. Two investigations in Shangai (China) identified occupational exposure to benzidine as the main cause of bladder cancer in some industrial settings. A retrospective cohort study in seven dyestuff factories" showed a standardized incidence ratio (SIR) of 3,500 **(14** observed cases) among 354 men in the presynthesis group,

Figure 1. Meta-analysis: N-acetyltransferase and bladder cancer.



Numbers in () are power of the study $\alpha = 0.05$, OR = 2)
g. = genotyping, ph. = phenotyping.
C = Caucasian., A = Asians.

Table 4. Correlation coefficients (Pearson) and *P*-values (in parentheses): urinary cotinine-nicotine, urinary 1-hydroxyprene, levels of 4-aminobiphenyl-hemoglobin adducts, and log DNA adducts in exfoliated bladder cells (39 healthy men) (from ref. 64)

Adduct No.	All subjects		Smokers ($n = 18$)	
	1-hydroxyprene	4-ABP	1-hydroxyprene	4-ABP
1	-0.01	0.06	0.02	0.09
	(0.95)	(0.70)	(0.92)	(0.72)
2	0.44	0.42	0.37	0.52
	(0.01)	(0.01)	(0.12)	(0.03)
3	-0.22	-0.03	-0.19	0.37
	(0.17)	(0.84)	(0.44)	(0.13)
4	0.02	0.33	0.01	0.54
	(0.90)	(0.04)	(0.97)	(0.02)
5	0.08	0.07	0.16	0.28
	(0.62)	(0.67)	(0.53)	(0.26)
6	-0.01	0.01	-0.08	0.09
	(0.93)	(0.96)	(0.74)	(0.09)
7	-0.10	-0.02	-0.17	-0.28
	(0.52)	(0.88)	(0.49)	(0.27)
8	-0.04	-0.10	–	–
	(0.77)	(0.53)		
9	-0.14	0.05	-0.22	-0.35
	(0.39)	(0.75)	(0.36)	(0.14)
10	-0.09	0.37	-0.15	0.34
	(0.58)	(0.02)	(0.55)	(0.16)
11	-0.03	-0.14	0.09	0.06
	(0.87)	(0.40)	(0.71)	(0.82)
12	0.14	-0.13	0.05	-0.17
	(0.38)	(0.42)	(0.84)	(0.48)
Total diagonal zone	0.09	0.17	0.13	0.45
	(0.57)	(0.29)	(0.61)	(0.06)

Abbreviations: 4-ABP = 4-aminobiphenyl-hemoglobin adducts.

and the SIR was up to 7,500 (six cases) in a subgroup of 69 subjects who mixed and transported benzidine. In a case-control study,[57] a significantly increased risk was observed (SIR = 167) for textile bleachers, dyers, and finishers. A case-control investigation in Bombay (India)[58] showed a fivefold bladder cancer risk (OR = 4.48, CI = **1.2-16.2)** among employees in companies manufacturing dyes.

Non-occupational sources of arylamines: tobacco smoking

Tobacco smoking is a well-known cause of bladder cancer accounting for more than 50 percent of bladder cancers in men and 20 percent in women, in Western societies and is a source or arylamines.[59] Air-cured (black) tobacco, is particularly rich in arylamines such as 4-amino-biphenyl; smokers of black tobacco have a risk of bladder cancer which is about 2.5-fold compared with smokers of flue-cured tobacco.[60] Studies of 'molecular' epidemiology have suggested that smokers of air-cured tobacco have higher levels of 4-aminobiphenyl-hemoglobin adducts (a marker of internal dose) in their blood, compared with smokers of flue-cured tobacco.[61] Biopsies of bladder cancer from smokers contain a DNA adduct identified as a derivative of 4-aminobiphenyl.[62] This same DNA adduct was present in exfoliated bladder cells of smokers;[63] the presence and concentration of the DNA adducts was correlated strongly with 4-aminobiphenyl-hemoglobin adducts but not with urinary 1-hydroxypyrene-glucuronide, a metabolite of benzopyrene,[64] as shown in Table 4; where the derivative of 4-arninobiphenyl is adduct **4.** The latter observation suggests that arylamines and not polycyclic aromatic hydrocarbons in tobacco smoke might be responsible for bladder cancer in smokers, but this is still an attractive hypothesis which warrants further evidence.

The concentration of 4-aminobiphenyl-hemoglobin adducts in both smokers and nonsmokers is modulated by the N-acetylation phenotype: irrespective of the smoking status of the subjects, the genetically based slow-acetylator phenotype was associated with higher concentrations of the adduct.[65] N-acetyltransferase deactivates carcino-

genic arylamines and has a genetically based polymorphic distribution in the population, with about 50 percent of Caucasians being 'slow' acetylators. Slow acetylators have been shown to be at high risk of bladder cancer in epidemiologic investigations.[66]

Figure 1 shows the individual investigations, and a meta-analysis of the studies, on the N-acetylator genotype or phenotype and bladder cancer. The consistency among the results obtained in different Caucasian populations and with different study designs seems to suggest that N-acetyltransferase exerts a causal role in modulating the risk of bladder cancer in arylamine-exposed subjects. An exception is represented by **two** studies in Asians, which show low RRs in slow acetylators.

Overall, epidemiologic observations suggest, as a working hypothesis, that arylamines such as 4-aminobiphenyl might be responsible for the excess risk of bladder cancer in smokers. In addition, the risk appears to be modulated by genetically-based metabolic polymorphisms such as N-acetyltransferase.

Gender differences

A difference between men and women in bladder cancer risks has been shown consistently in epidemiologic studies. For tobacco smoking,[59] most studies documented a positive association, but with RRs for women generally lower than for men. Some investigations also have shown a lower RR in women after allowing for differences in exposure to specific risk factors.

In the cohort analysis of lung and bladder cancer mortality in Denmark, 1943-87,[67] both genders had the same overall lung cancer risk, while for bladder cancer, women showed a smaller increase associated with smoking compared with men (3.7 *cf* 6.1-fold). A case-control study (2,806 cases and 5,258 controls) in the US[68] showed, in 1978, a male/female ratio of 2.7 after adjustment for occupational exposures and cigarette smoking. An incident case-control study carried out in Orange County, California (US) for the years 1984-87[69] showed that the OR for men relative to women was 5.95 (CI 4.36-8.12), after adjustment for age, ethnicity, smoking status, and occupation. Therefore, the male/female ratio in bladder cancer is not explained completely by gender differences in smoking habits or occupational exposures. A case-control study in the US using mailed questionnaire showed a decreased risk for parous *cf* nulliparous women (OR = 0.67, CI = 0.44-1.0), the reduction in risk being restricted to never-smokers (OR = 0.51, CI = 0.30-0.88). There was no apparent effect of parity in nonsmokers (OR = 0.93). A further hint, that suggests genuine biological differences between genders, is the different spontaneous background frequency of micronuclei in exfoliated bladder cells in men and women.[71]

Conclusions

According to the Working Groups of the IARC Monographs Program, seven arylamines have been classified as carcinogenic to humans (Group 1) or 'probably' carcinogenic to humans (Group 2A). In addition to three specific occupational chemicals (2-naphthylamine, benzidine and MOCA), and one drug [N,N-bis(2-chloromethyl)-2-naphthylamine], Chlornaphazine), also one group of industrial compounds (benzidine-based dyes, *i.e.*, Direct Black 38, Direct Blue **6** and Direct Brown 95), and two manufacturing processes (manufacture of auramine and magenta) have been listed. Whereas for the other chemicals or industrial processes, the evidence of carcinogenicity in humans was sufficient, benzidine-based dyes and MOCA were considered 'probably' carcinogenic because of a high level of evidence in experimental animals.

Occupational exposures to aromatic amines explain up to 25 percent of bladder cancers occurring in some areas of Western countries. Estimates of the attributable fraction are strictly space- and time-specific, and might be higher in limited areas of developing countries. Aromatic amines contaminate the ambient air as a component of environmental tobacco smoke. There is increasing evidence that the excess of bladder cancer in smokers is attributable to aromatic amines rather than to other contaminants of tobacco smoke such as PAHs. A modulating role in the risk of bladder cancer associated with exposure to aromatic amines is played by metabolic polymorphisms, such as the N-acetyltrasferase genotype. Greater susceptibility of slow acetylators to the carcinogenic effect of aromatic amines raises important social and ethical issues that have been addressed elsewhere." The consistent observation of a difference between men and women in bladder cancer risk, after allowing for known risk factors, suggests consideration of gender-related biological determinants for future investigation.

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