Epidemiology of Cancer from Exposure to Arylamines

Paolo Vineis

Unit of Cancer Epidemiology, Dipartimento di Scienze Biomediche e Oncologia Umana, Torino, Italy

Occupational exposure to arylamines such as benzidine, 2-naphthylamine, and 4-aminobiphenyl is associated with exceptionally elevated risks of bladder cancer (up to 100-fold or more). In one plant, all 15 workers involved in distilling naphthylamine developed bladder cancer, suggesting that for high levels of exposure to potent carcinogens individual susceptibility is irrelevant. More recently, exposure to other arylamines also has been suggested to increase the risk of bladder cancer in humans. In addition, cohort and case–control studies suggest that several job titles or exposures may involve elevated risks of bladder cancer. Some of these jobs or exposures (such as in the aluminum industry) are associated with exposure to arylamines. Arylamines are found also in tobacco smoke, and different sources of evidence suggest that they can explain the risk of bladder cancer, which has been shown clearly in smokers. Epidemiologic analyses of timing of exposure in workers occupationally exposed to arylamines or in air-cured tobacco smokers suggest that arylamines exert both an early- and a late-stage activity, compatible with a two-mutation theory of bladder carcinogenesis. — Environ Health Perspect 102(Suppl 6):7–10 (1994)

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Occupational Exposure to Defined Carcinogenic Arylamines

According to the working groups of the International Agency for Research on Cancer Monographs Programme, 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), one drug (Chlornaphazine), 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 (*1*). 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.

Following anecdotal reports and experimental work in dogs (*2*), the carcinogenicity of arylamines such as 2-naphthylamine and benzidine was clearly demonstrated in the 1950s in a well designed epidemiologic investigation on British chemical workers (*3*). Case and colleagues were able to obtain from the chemical industry nominal rolls for all workers, including information on their job titles and the chemicals they manufactured. Overall, 4622 men in 21 firms were enrolled, and mortality from bladder cancer was ascertained. The observed deaths exceeded by far those expected among workers exposed to 2-naphthylamine and benzidine, while lower excesses were reported for aniline and 1-naphthylamine (Table 1). However, subsequent studies did not confirm that the latter substances were carcinogenic, and the apparent excess was probably because of contamination from 2-naphthylamine (*1*). The average induction period of bladder cancer after exposure to 2-naphthylamine and benzidine was 16 yr (*3*). The peculiar conditions that made the study by Case and colleagues feasible should be stressed: the availability of nominal rolls with detailed knowledge of individual chemical exposure, including timing, is in fact rather unusual in occupational epidemiology. In addition, potent carcinogens were investigated, and this entailed high-statistical power to detect a causal association.

Other studies have reported considerable increased risks of bladder cancer in workers exposed to 2-naphthylamine, benzidine, and 4-aminobiphenyl (Table 2). The different entity of risk ratios probably reflects different levels of exposure. Important evidence strengthening the cause–effect relationship comes from the observation that cessation of exposure to benzidine was followed by a decline of the incidence of bladder cancer (*4*).

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 (*5*).

In addition to the arylamines shown in Table 2, recent evidence suggests *o*-toluidine as a bladder carcinogen. In their study, Rubino et al. (*6*) found a 62-fold

Table 1. Risk of bladder cancer associated with specific chemicals from the dye industry (*3,17*).

Chemical	Observed deaths from bladder cancer	Expected deaths	Ratio
Aniline	4	0.9	4.5
Benzidine	10	0.7	14
1-Naphthylamine	6	0.7	9
2-Naphthylamine	26	0.3	87
Mixed exposures	81	1.5	55
All classes	127	4.1	31

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Address correspondence to Paolo Vineis, Unit of Cancer Epidemiology, Dipartimento di Scienze Biomediche e Oncologia Umana, via Santena 7, 10126 Torino, Italy. Telephone 11 6706525. Fax 11 6635267.

increase in bladder cancer risk in workers exposed jointly to *o*-toluidine and 4,4′-methylene bis(2-methylaniline) [both carcinogenic to experimental animals (*1*)]. The excess was based on five cases. Stasik reported a 72-fold increase (eight cases) among workers exposed both to *o*-toluidine and to 4-chloro-*o*-toluidine (*7*). More recently, a study conducted by the National Institute for Occupational Safety and Health (NIOSH), concerning a cohort of workers exposed jointly to *o*-toluidine and aniline, found high relative risks (*8*) (Table 3). Unfortunately, none of these investigations enable us to evaluate separately the contribution of *o*-toluidine to the excess of bladder cancer. *o*-Toluidine is clearly carcinogenic in experimental animals, whereas the data are less convincing for aniline (*1*). Finally, in a group of 49 workers in Germany, 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 (*9*).

Table 2. Arylamines with clear evidence of carcinogenity to humans (*1,18,19*).

Study	Number of observed deaths	O/E[a]
2-Naphthylamine		
Case et al., 1954	26	87
Mancuso & El-Attar, 1967	18	30
Goldwater et al., 1965	12[b]	—
Schulte et al., 1985	13[c]	3.9
Rubino et al., 1982	6	150
Benzidine		
Case et al., 1954	10	14
Mancuso & El-Attar, 1967	16	30
Zavon et al., 1973	13[d]	—
Tsuchiya et al., 1975	72[e]	—
Rubino et al., 1982	5	83
Horton et al., 1977	13[f]	—
Meigs et al., 1986	6[g]	130
Hayes et al., 1992	31	25
4-Aminobiphenyl		
Melick et al., 1971	53[h]	—
Zack & Gaffey, 1983	9	10

[a]Observed/expected ratio. [b]Among 48 workers. [c]Incident cases. [d]Among 25 workers. [e]Among 1015 workers. [f]Among 25 workers. [g]High level of exposure. [h]Among 315 workers.

Table 3. Incidence of bladder cancer among workers exposed to *o*-toluidine and aniline in the National Institute for Occupational Safety and Health study (*8*).

	Risk ratio
All workers	3.6[a] (based on 13 cases)
Definitely exposed	6.5[a] (7 cases)
Possibly exposed	3.7[a] (4 cases)
Probably unexposed to *o*-toluidine and aniline	1.4 (2 cases)
Only definitely exposed workers at the plant:	
< 5 years	0
5–10 years	8.8
10 + years	27.2[a]

[a]$p < 0.05$.

Other Relevant Epidemiologic Evidence

One of the major limitations of many epidemiologic studies is the lack of detailed information on exposure to individual chemicals. Nevertheless, epidemiologic studies of the cohort or case-referent type suggest associations between several job titles or industrial activities and the risk of bladder cancer. It is not possible to summarize all these associations, which warrant further investigation to ascertain the role of specific chemicals. However, a few of these clues are worth mentioning because of the consistency of the association, the high prevalence of exposure, and the possible contamination of exposure by arylamines (*10*). One is represented by jobs with exposure to combustion gases and soot from coal (Table 4). In addition to polycyclic aromatic hydrocarbons, such jobs involve exposure to arylamines. For example, in a cohort study of coal carbonizing workers, 2-naphthylamine was found in a sample from tar volatiles (*11*). One of the most interesting investigations was conducted by Theriault and colleagues (*12*) in which they found in the aluminum industry considerably elevated relative risks (up to 12) after documented exposure to benzo[*a*]-pyrene. They could not rule out that exposure to arylamines also occurred, as more recent evidence suggests [Tremblay, personal communication; (*10*)]. Another type of exposure with likely contamination by arylamines (e.g., phenyl-*a*-naphthylamine) is exposure to cutting oils and cutting fluids where excess risks of bladder cancer have been shown repeatedly (*13*).

The Contribution of Occupational Exposure to Bladder Cancer

A legitimate question is: how many bladder cancers are attributable to occupational

Table 4. Combustion gases and soot from coal: relative risk of bladder cancer in epidemiologic studies (*10*).[a]

Author, year	Title and exposure	Relative risk	95% CI
Cohort studies			
Doll, 1972	Coal carbonizers	2.4	1.1–4.3
Redmond, 1972	Coke oven workers	1.2	0.1–4.2
Hammond, 1976	Roofers and waterproofers: duration		
	9–19 years	0.8	0.1–3.0
	20+ years	1.7	0.9–2.9
Gustavsson, 1988	Chimney sweeps	2.3	1.4–3.4
Steineck, 1988	Combustion gases from coal	1.2	1.0–1.4
Case referent studies, population-based			
Howe, 1980	Glass processors	6.0	0.7–276
McLaughlin, 1983	Coal/natural gas	2.9	1.0–8.2
	Soot	3.0	0.9–8.9
Silverman, 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 manufacturerers	5.9	0.7–50
Mommsen, 1984	Blacksmiths	5.0	0.6–236
Schoenberg, 1984	Cooks	1.3	0.8–2.3
Theriault, 1984	Documented exposure to benzopyrene: duration		
	10–19 years	6.8	2.6–17.8
	20+ years	12.4	3.3–46.1
Morrison, 1985			
England	Cooks	1.0	0.7–1.6
Japan	Cooks	1.0	0.4–2.4
United States	Cooks	1.2	0.7–2.1
England	Coal, coke	0.8	0.6–1.3
Japan	Coal, coke	1.3	0.6–2.6
United States	Coal, coke	1.1	0.5–2.0
Case referent studies, hospital-based			
Dunham, 1968	Smiths	7.6	0.4–128
Tola, 1980	Smiths	0.4	0.1–1.2
	Foundry workers	2.0	
Vineis, 1985	Brickyards	2.0	0.9–4.5
	Foundries	0.7	0.4–1.3

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

exposure to arylamines? The answer is quite uncertain because we know very few occurrences in which well-documented exposure to arylamines was associated with increased risk of bladder cancer. In many other investigations, particularly of the case-referent type, we only measure—sometimes consistently across studies—increased risks for job titles without knowledge of arylamine exposure. An attempt to measure the population attributable risks (PAR) was made recently using evidence from published case–control studies that adjusted the estimates by smoking habits (*14*). In all the PAR estimates, we have included workers of dye-producing plants, rubber workers, and gas workers under the assumption that they were exposed to arylamines. In addition, we have used three criteria to include job titles into the PAR estimates. According to the first criterion (the less stringent), we included the job titles in all the published studies associated to bladder cancer risk (odds ratio greater than 1.0) and showing a statistically significant association in at least one study (Table 5, criterion I). According to the second criterion, a further condition was that the proportion of exposed controls was at least 5%. According to the most stringent criterion, the same conditions applied, but only associations that reached statistical significance in at least two studies were considered (criterion III) (*14*). As one can easily see, the PAR estimates vary 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.

The reported estimates suffer from several limitations: *a*) the classification of exposed workers is based on job titles and not on actual knowledge of chemical exposure; *b*) the classification of workers within job categories is affected by errors that tend to entail underestimation of risks; *c*) different studies have been conducted with different methods and different classification schemes. Nevertheless, our 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%.

Table 5. Risk of bladder cancer attributable to occupational exposures in different studies and according to different criteria.[a]

Study	Attributable risk %		
	Criterion I	II	III
Wynder et al., 1963	0	3	3
Anthony and Thomas, 1970	3	7	7
Cole et al., 1972	10	18	16
Howe et al., 1980	7	5	4.5
Tola et al., 1980	4	5	5
Cartwright, 1982	19	21	20
Silverman et al., 1983	19	19	15
Schoenberg et al., 1984	9	10	3
Vineis and Magnani, 1985	6	10	9.5
Iscovivh et al., 1987	18	14	14
Jensen et al., 1987	5	11	11
Schifflers et al., 1987	17	17	16
Claude et al., 1988	18	13	13
Gonzales et al., 1989	6	11.5	10
Schumacher et al., 1989	2	2	2
Bonassi et al., 1989	24	24	24
Silverman et al., 1989	7	7	6
Silverman et al., 1989	8	8.5	2.5

[a]Criterion I includes rubber workers, dyestuff production, gas workers, petroleum workers, machinists and engineers, truck drivers, garage and gas station workers, and food counter workers and cooks. Criterion II includes the same except petroleum workers, and in addition textile workers, printers, and leather workers. Criterion III includes only textile, rubber, dyestuff, leather workers, truck drivers, machinists, and engineers (attributable risk percent) (*14*).

Nonoccupational Sources of Arylamines: Tobacco Smoking

Tobacco smoking is a source of arylamines (*15*). Briefly, a few studies of traditional and molecular epidemiology have suggested: *a*) the type of tobacco that has been associated with the highest risks of bladder cancer (air-cured tobacco) is also richer in arylamines (*15,16*); *b*) smokers of air-cured tobacco have higher levels of 4-aminobiphenyl-hemoglobin adducts (a marker of internal dose) in their blood compared to smokers of flue-cured tobacco; *c*) biopsies of bladder cancer from smokers contain a DNA adduct identified as a derivative of 4-aminobiphenyl; *d*) the same DNA adduct was present in exfoliated bladder cells of smokers; and *e*) the concentration of 4-aminobiphenyl-hemoglobin adducts in both smokers and nonsmokers was modulated by the *N*-acetylation phenotype. The latter observation is shown in Table 6; irrespective of the smoking status of the subjects, the genetically based slow-acetylator phenotype was associated with higher concentrations of the adduct (*16*). Overall, these epidemiologic observations suggest that arylamines such as 4-aminobiphenyl might be responsible for the excess risk of bladder cancer in smokers.

Timing of Exposure and the Risk of Bladder Cancer

The mechanisms of carcinogenesis are an important subject for investigation, and epidemiology can contribute through the study of timing of exposure and cancer onset. Hoover and Cole originally observed that the risk of occupational bladder cancer was higher in workers exposed at younger ages (*17*). Detailed analyses of a case–control study and a cohort study, both conducted in the province of Torino, Italy, revealed that the relationship between timing of exposure and the risk of bladder cancer was very similar after exposure to air-cured tobacco and to occupational arylamines (Table 7). The risk decreased with increasing time since first exposure and decreased with increasing time since exposure cessation. The trends were almost overlapping for the two exposures, further suggesting that the bladder carcinogenicity of air-cured tobacco may be attributed to arylamines. The fact that both age at start and cessation of exposure have an influence in modifying the relative risk of cancer is interpreted as to suggest that two stages in the mechanism of bladder carcinogenicity are involved (*16*), one early and one late. This model is consistent with a two-mutation hypothesis of bladder carcinogenesis.

Table 6. Means and SEs of 4-aminobiphenyl hemoglobin adducts in the blood of air-cured and flue-cured tobacco smokers and nonsmokers (pg/g Hb) by acetylation phenotype (*16*).

	Acetylation phenotype	
	Slow	Fast
Nonsmokers	31.7 (3.8)	19.4 (4.9)
Flue-cured tobacco	111.8 (13.0)	86.4 (14.5)
Air-cured tobacco	175.0 (11.0)	117.5 (13.7)

Table 7. Relative risks by age at start and years since cessation, among air-cured tobacco smokers and a cohort of workers occupationally exposed to arylamines (*16*).

	Age at start				Time since cessation, years			
Air-cured tobacco[a]	<17	17–20	21–24	25+	<3	3–9	10+	
	1.0	0.4	0.4	0.3	1.0	0.45	0.27	
Occupational exposure	<25	25-34	35+		0	<10	10–19	20+
	1.0	0.4	0.2		1.0	0.4	0.2	0.15

[a]Estimates adjusted for age and number of cigarettes smoked.

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