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Mortality from bladder cancer in dyestuff workers exposed to aromatic amines: A 73-year follow-up

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Abstract

Objective: *To update the analysis of mortality of a cohort of dyestuff workers, in northern Italy, heavily exposed to carcinogenic aromatic amines.* **Methods:** *We updated to 2018 overall and cause-specific mortality in a cohort of 590 male workers heavily exposed to carcinogenic aromatic amines in a dyestuff factory from 1922 to 1972. Workers were censored at age 85. Expected cases for the period 1946-2018 were computed using Piedmont mortality rates and standardized mortality ratios (SMR) were computed.* **Results:** *Between 1946 and 2018, 470 deaths were reported. The overall SMR from all causes was 1.59 (95% confidence interval [CI] 1.45-1.74) and the SMR from all cancers was 2.05 (95% CI = 1.77-2.37); compared to a previous report, there were 4 additional deaths from bladder cancer, for a total of 60 deaths compared with 4.0 expected (SMR 14.86, 95% CI 11.34-19.12). The SMR for bladder cancer increased with younger age at first exposure and longer duration of exposure, while it decreased with time since last exposure, albeit it was still 3.5, 30, or more years since last exposure. An increased risk was observed among workers exposed to fuchsine or ortho-toluidine (SMR=16.3; 95% CI = 6.0-35.5).* **Conclusions:** *This 73-year follow-up confirms the results from previous analyses, with increased overall mortality, and increased mortality from all cancers and especially for bladder cancer. The excess risk of bladder cancer persisted several decades after stopping exposure.*

1. Introduction

Bladder cancer is associated with substantial morbidity, mortality, and cost. It is the 10th most commonly diagnosed cancer worldwide, with approximately 573,000 new cases/year and 213,000 deaths [1]. Incidence in men is about three times higher compared to women. The disease ranks higher among men, for whom it is the 6th most common cancer [2].

Tobacco smoking is the main risk factor for bladder cancer accounting for 50-65% of all cases [3]. Occupational or environmental carcinogens also contribute to disease burden, though the precise proportion can be obscured by the fact bladder cancer develops decades after exposure [4]. In industrialized countries, in the period from 1978 to 2012, about 2-5% of all cancers [5-7] and 5–6% of all bladder cancers [8] were considered to be caused by occupational factors.

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Prevention measures contributed to reducing the burden of occupationally related cases of this tumor [9]. A study that reviewed 263 papers on occupational bladder cancer [10] found that the highest pooled incidence risks are for tobacco workers (RR 1.72 [95% CI 1.37–2.15]) and dye workers (RR 13.4 [95% CI 1.5–48.2]), while the highest pooled disease-specific mortality was in metal workers (RR 10.2 [95% CI 6.89–15.09]).

In 1895 the surgeon Ludwig Rehn firstly described 3 cases of occupational bladder tumors in at most 45 fuchsine workers in Frankfurt [11]. There were also early reports of an increased risk of bladder cancer in workers employed in the aromatic amine industry [12], still, the production of these agents continued till the 1970s.

Rubino et al [13] published a hospital-based study showing 23 cases of malignant bladder neoplasms among workers of a dyestuff factory in the province of Turin (northern Italy) who were heavily exposed to α-naphthylamine, β-naphthyl amine, benzidine, and other chemicals including ortho-toluidine, up to 1970. That cohort study was one of the first studies to support the hypothesis that ortho-toluidine was a human bladder carcinogen.

The cohort study and the subsequent updates [14- 17] confirmed a substantial increased total mortality and mortality from bladder cancer. Analyses by time since cessation of exposure showed increased risk 20 years or more after exposure stopped.

Through an update of the follow-up including an additional 15 years of observation of this cohort, we aimed at providing unique information on bladder cancer risk after long-term cessation of exposure to a high level of carcinogenic aromatic amines.

2. Methods

The cohort comprised only men who had worked for at least one year in the factory between 1922 and 1970 and were exposed to aromatic amines. No women were employed in production jobs in the factory. White collars were not included in the cohort. Information on date of birth, date of first and last employment, last known address, and detailed job history (including exposure to selected chemicals) were obtained from personnel records at the plant.

The follow-up for mortality was conducted by contacting the municipality of residence; death certificates were obtained from the same source and causes of death were coded according to the 9th Revision of the International Classification of Diseases. The follow-up was truncated when cohort members reached the age of 85. For the present analysis, the follow-up began on 1st January 1946 or date of first employment, whichever occurred later, and ended on the date of last available information on known address or vital status, date of death, 85th birthday, or 30 November 2018, whichever occurred earlier. Truncation of follow-up at the 85th birthday is justified by the lower quality of death certification above that age.

The cohort comprised 590 men that met the inclusion criteria (18626 men-years), as they were employed in the production of dyestuff, for at least one year, between 1922 and 1970, and alive on January 1st, 1946.

At the end of the follow-up, 30 November 2018, 93 subjects (15.8%) were alive, 470 (79.7%) were deceased, 26 of whom from unknown causes, and 27 (4.6%) were lost to follow-up.

Exposure classification is reported in Table 1. As described by Rubino et al. [14] workers involved in α-naphthylamine manufacture, β-naththylamine manufacture, benzidine manufacture, and/or in mixed manufacture of benzidine and naphthylamine were grouped into the high exposure category for this analysis (Group A).

Table 1. Exposure classification

Group	Exposure
Group A	α-naphthylamine manufacture, β-naththylamine manufacture, benzidine manufacture, and mixed manufacture of benzidine and naphthylamine
Group B	workers never involved in the manufacture, but only in the use of naphthylamine and benzidine
Group C	workers with intermittent contact with naphthylamine and benzidine
Group D	workers who were involved only in the manufacture of fuchsin or o-toluidine
Group E*	workers employed in other jobs not involving exposure or unknown exposure

** Excluded from the analysis*

Group B included the workers never involved in the manufacture, but only in the use of naphthylamine and benzidine, in group C workers with intermittent contact with naphthylamine and benzidine while in group D there were workers only involved in the manufacture of fuchsin or o-toluidine.

A further group (Group E) included workers employed in jobs with no exposure to aromatic amines or with unknown exposure to aromatic amines. Workers in group E were excluded from the analysis.

The number of expected deaths from all causes and selected causes was computed using male national and regional (from 1981 onwards) death rates for each 5 years calendar period and age group.

For the period 1946–1950, we used rates of 1951–1954. Because retirement or change of job may in some cases be caused by bladder cancer, and for consistency with our previous reports [14- 17], deaths that occurred within 3 years of stopping exposure were considered with those occurring during exposure.

From the numbers of observed and expected deaths, we calculated the standardized mortality ratios for selected causes of death and their corresponding 95% confidence intervals (CIs) by use of the Poisson distribution for observed deaths [18]. Tests for linear trends were also based on the Poisson statistics proposed by Armitage [18, 19].

3. Results

A total of 470 cohort members died during the follow-up. There were also 42 deaths that occurred in subjects older than 85 that were not included in the analysis. Results of the mortality follow-up, for all causes and specific causes of death, are reported in Table 2.

This update confirms increased overall mortality (SMR 1,59; 95% CI = 1.45-1.74), and increased mortality from all cancers (SMR 2.05; 95% CI = 1.77-2.37). A total of 60 deaths were caused by bladder cancer (4.0 expected; SMR 14.86; 95% CI = 11.34-19.12).

Excess mortality was observed for alcohol-related cancers, including oral and pharyngeal cancer (SMR = 2.46, 95% CI = 0.99-5.08), esophageal cancer (SMR = 2.23, 95% CI = 0.72-5.20), colorectal cancer (SMR = 2.09, 95% CI = 1.28 to 3.23), liver cancer (SMR = 1.54, 95% CI = 0.56-3.35), laryngeal cancer (SMR = 3.92, 95% CI = 1.95-7.01) and for other alcohol-related causes including liver cirrhosis (SMR = 3.70, 95% CI = 2.68-4.98), and external causes or injuries (SMR = 1.27, 95% CI = 0.79 to 1.92).

Mortality from lung cancer was not significantly increased (SMR = 1.24, 95% CI = 0.84-1.86). There were 4 deaths from pleural cancer and 2 deaths from peritoneal cancer. Two of these workers had started working in the plant after the age of 35.

Results of the mortality analysis from bladder cancer, all other cancers, and all causes based on time since last employment, age at first exposure, and duration of exposure are presented in Table 3.

There were 16 observed deaths for bladder cancer during employment (SMR for bladder cancer = 59.7, 95% CI = 34.1-97.0), 15 between 1 and 9 years since last exposure (SMR = 29.3, 95% CI = 16.4-48.3), 13 between 10 and 19 years since last exposure (SMR = 14.5, 95% CI = 7.7-24.9), 11 between 20 and 29 years since last exposure (SMR = 12.0, 95% CI = 6.0-21.6), and 5 for 30 years or more since last exposure (SMR = 3.5, 95% CI = 1.1-8.0, test for linear trend, $p<0.0001$).

Furthermore, the risk of bladder cancer was higher in workers exposed for 10 years or more as compared with those exposed for less than 10 years (p for trend=0.001). For other cancers and deaths overall, no consistent pattern of risk with time since last exposure, age at first exposure, or duration of exposure was observed.

Results of mortality from bladder cancer by job categories are presented in Table 4. We observed 35 bladder cancer deaths among workers belonging to group A (SMR = 45.4, 95% CI = 31.6-63.1), 8 bladder cancer deaths in group B (SMR = 8.8, 95% CI = 3.8-17.3), and 11 bladder cancer deaths in group C (SMR = 5.5, 95% CI = 2.8-9.9).

There was also excess mortality for bladder cancer among the group D workers, manufacturers of fuchsin or ortho-toluidine (Observed = 6 deaths, SMR = 16.3, 95% CI = 6.0-35.5).

4. Discussion

This updated mortality analysis of a cohort of dyestuff workers provides for the first time strong

Table 2. Numbers of observed and expected deaths from selected causes, 1946-2018

Cause of death	Obs	Exp	SMR (95%CI)
All causes	470	296.5	1.59 (1.45-1.74)
All cancers	187	91.1	2.05 (1.77-2.37)
Oral and pharyngeal cancer	7	2.8	2.46 (0.99-5.08)
Esophageal cancer	5	2.2	2.23 (0.72-5.20)
Stomach cancer	10	10.1	0.99 (0.47-1.82)
Colorectal cancer	20	9.6	2.09 (1.28-3.23)
Liver cancer	6	3.9	1.54 (0.56-3.35)
Pancreas cancer	4	3.4	1.18 (0.32-3.02)
Peritoneal cancer	2	0.4	4.60 (0.56-16.60)
Laryngeal cancer	11	2.8	3.92 (1.95-7.01)
Lung cancer	31	25.1	1.24 (0.84-1.86)
Pleural cancer	4	0.8	5.29 (1.44-13.55)
Prostate cancer	6	5.5	1.09 (0.40-2.37)
Bladder cancer	60	4.0	14.86 (11.34-19.12)
Kidney cancer	2	1.8	1.11 (0.13-3.40)
Brain & CNS cancers	1	2.1	0.47 (0.01-2.60)
Lympho-hematopoietic neoplasms	5	5.9	0.85 (0.28-1.98)
Lymphomas	3	2.4	1.25 (0.26-3.66)
Myeloma	0	1.0	0 (0-3.00)
Leukemias	2	2.5	0.79 (0.10-2.84)
Ischemic heart diseases	29	43.6	0.67 (0.45-0.96)
Cerebrovascular diseases	31	32.5	0.95 (0.65-1.36)
Non neoplastic resp. dis.	29	22.3	1.30 (0.87-1.87)
COPD	20	13.2	1.52 (0.93-2.34)
Pneumoconiosis	0	2.1	0 (0-1.43)
Liver cirrhosis	43	11.6	3.70 (2.68-4.98)
External causes	22	17.4	1.27 (0.79-1.92)

CI, confidence interval; CNS, central nervous system; COPD, Chronic Obstructive Pulmonary Diseases; Exp, exp0ected deaths; ICD-IX, International Classification of Diseases, 9th version; Obs, observed deaths; SMR, standardized mortality ratio.

evidence that high-level exposure to carcinogenic aromatic increases the risk of bladder cancer even after three or more decades from the cessation of exposure. With 87% (512 subjects) of the members of the cohort deceased during a 73-year follow-up and with almost 50 years of follow-up since the factory ceased the activity, this cohort provides unique results on long-term health effects of workers exposed to high levels of carcinogenic aromatic amines. In fact, 32% of all cancer deaths were from bladder cancer, compared to 6% among Italian men or 4% among US men [20].

Strengths of our study include the uniquely long follow-up that allowed separate analyses by latency and other time-related factors. Also, the availability of data on exposure type and exposure levels in the early period of operation of the plant was already reported in the previous follow-ups [13, 14]. This updated analysis showed the persistence of increased bladder cancer risk long after cessation of exposure.

Table 3. Mortality from bladder cancer, all other cancers, and all causes by time since last exposure, age at first exposure, and duration of exposure, 1946- 2018

CATEGORY	BLADDER CANCER			ALL CAUSES		
	Obs	Exp	SMR (95%CI)	Obs	Exp	SMR (95%CI)
Time since last exposure						
During exposure	16	0.3	59.7 (34.1-97.0)	57	38.3	1.5 (1.1-1.9)
1-9 years	15	0.5	29.3 (16.4-48.3)	99	51.4	1.9 (1.6-2.3)
10-19 years	13	0.9	14.5 (7.7-24.9)	101	68.4	1.5 (1.2-1.8)
20-29 years	11	0.9	12.0 (6.0-21.6)	88	58.0	1.5 (1.2-1.9)
≥ 30 years	5	1.5	3.5 (1.1-8.0)	125	80.4	1.6 (1.3-1.9)
p trend			<0.001			0.6
Age at first exposure						
< 25 years	19	1.1	17.3 (10.4-27.0)	115	72.9	1.6 (1.3-1.9)
25-34 years	18	1.3	14.0 (8.3-22.1)	135	85.6	1.6 (1.3-1.9)
≥ 35 years	23	1.7	14.0 (8.8-21.0)	220	138.0	1.6 (1.4-1.8)
p-trend			0.5			1.0
Duration of exposure						
< 10 years	16	2.1	7.6 (4.3-12.4)	251	153.0	1.6 (1.4-1.9)
10-19 years	22	0.9	24.6 (15.4-37.3)	120	72.5	1.7 (1.4-2.0)
≥ 20 years	22	1.0	21.1 (13.2-32.0)	99	70.9	1.4 (1.1-1.7)
p-trend			0.001			0.4

CI, confidence interval; Exp, expected; Obs, observed; SMR, standardized mortality ratio.

Table 4. Mortality from bladder cancer by job category, 1946- 2018

JOB CATEGORY	Obs	Exp	SMR (95%CI)
Group A: Naphthylamine and benzidine manufacture	35	0.8	45.4 (31.6-63.1)
Group B: Naphthylamine and benzidine use	8	0.9	8.8 (3.8-17.3)
Group C: Intermittent contact with naphthylamine and benzidine	11	2.0	5.5 (2.8-9.9)
Group D: Fuchsine or ørtho-toluidine manufacture	6	0.4	16.3 (6.0-35.5)

CI, confidence interval; Exp, expected; Obs, observed; SMR, standardized mortality ratio.

The excess risk was greater for workers directly involved in the manufacture of naphthylamine, benzidine, fuchsine, or ortho-toluidine than for those with intermittent exposure.

Limitations include the lack of direct information on potential confounders including lifestyle habits (e.g., alcohol drinking, tobacco smoking) and individual environmental exposure data. Data on tobacco smoking and alcohol drinking can be only indirectly extrapolated from the excess mortality for diseases strongly associated with these factors, such as lung cancer, chronic obstructive pulmonary disease, and liver cirrhosis. We found no relation with time since first or last exposure or with the duration of exposure for any of these causes of death. Therefore, it is plausible that the excess mortality from those causes is a result of heavy alcohol drinking, tobacco smoking, and other lifestyle characteristics rather than occupational exposure.

The lack of validation of causes of death from death certificates and the lack of information on morbidity may also represent limitations of the study,

although these potential sources of bias are unlikely to contribute to the excess bladder cancer mortality. We excluded workers with less of one year of employment, but, for sake of comparison with previous publications [14, 16,17] we did not exclude the first year of follow-up of the other workers. While this approach would inflate the number of person-years (for a maximum of 590 person-years, 3.2% of the total), the additional person-years were accumulated at a relatively young age, thus contributing little to the expected number of deaths.

We do not have more specific medical information about the 4 deaths from pleural cancer and the 2 deaths from peritoneal cancer (Supplementary Table 1). We tried to retrieve individual data through the Regional Operational Center of the National Mesothelioma Registry [https://www.cpo.it/it/home/].

However, after repeated attempts, we have not been able to obtain any information on the matter as bureaucratic hurdles arose at each step. The information from death certification and the proximity to the chrysotile asbestos mine of Balangero [21] allows us to formulate possible hypotheses: first, 2 of the 6 cases, had been previously employed in jobs other than that in the dyestuff factory, therefore, previous occupational exposure to asbestos cannot be excluded; second, residence near the mine is another possibility; finally, while we do not have information about the presence of asbestos-containing materials in the dyestuff factory, this can be another possible hypothesis. The six workers with mesothelial tumor as cause of death, worked in the factory for a median time of 6 years, with a median latency of 40.5 years (Supplementary Table 1).

5. Conclusions

In conclusion, this study provides evidence of the persistence of a substantial excess risk of bladder cancer among dyestuff workers with heavy exposure to aromatic amines, more than three decades after cessation of exposure.

Declaration of Interest: The authors declare no conflict of interest.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*.2021; 71:209–49. PMID: 33538338.
2. Godono A, Clari M, Franco N, et al. (2021). The association between occupational asbestos exposure with the risk of incidence and mortality from prostate cancer: A systematic review and meta-analysis. *Prostate Cancer and Prostatic Diseases*, online ahead of print. PMID: 34413482.
3. Sanli O, Dobruch J, Knowles MA, et al. Bladder cancer. Nat Rev Dis Primers. 2017;3:17022. PMID: 28406148.
4. Saginala K, Barsouk A, Sukumar Aluru J, Rawla P, Anand Padala S, Barsouk A. Epidemiology of Bladder Cancer. *Med Sci* (Basel) 2020;8(1):15. PMID: 32183076.
5. Pira E, Garzaro G, Ciocan C, Boffetta P. (2020) Occupational Cancer in the Practice of Occupational Medicine. In: Anttila S, Boffetta P. (eds) Occupational Cancers. Springer, Cham. https://doi.org/10.1007/978-3-030-30766-0_36E.
6. Purdue MP, Hutchings SJ, Rushton L, Silverman DT. The proportion of cancer attributable to occupational exposures. Ann Epidemiol. 2015; 25(3): 188–192. PMID 25487971.
7. Pira E, Coggiola M, Ciocan C, et al. Response to Letter to the Editor On the Mortality of Talc Miners and Millers From Val Chisone, Northern Italy. *J Occup Environ Med*. 2017;59(10):e195. PMID 28991140.
8. Westhoff J, de Oliveira-Neumayer m, Aben KK, Vrieling ., Kiemeney LA . Low awareness of risk factors among bladder cancer survivors: New evidence and a literature overview. *Eur J Cancer*. 2016; 60: 136-45. PMID: 27125965.
9. Pira E, Garzaro G, De Cilli, E, Donato F, Ciocan C, Patrucco M. Evolution of the concept OS&H from the second post-war to today: From prescriptive system to assessment and management of risks in system quality -the extended model in collaboration with large facilities. evolution of multidisciplinary culture of safety and OS&H. [Evoluzione del concetto di OS&H dal secondo dopoguerra ad oggi: Dal sistema prescrittivo alla Valutazione e Gestione dei rischi in qualità di sistema - il modello esteso in collaborazione alle grandi strutture. Evoluzione della cultura multidisciplinare della sicurezza e OS&H]. *Geoingegneria Ambientale e Mineraria*. 2018;154(2):16-20. Retrieved from www.scopus.com.
10. Cumberbatch MGK, Cox A, Teare D, Catto JWF. (2015) Contemporary occupational carcinogen exposure and bladder cancer: a systematic review and meta-analysis. *JAMA Oncol*. 2015; 1: 1282-1290. PMID: 30268659.

11. L. Rehn. Blasengeschwülste bei Fuchsin-Arbeitern. *Verh Dtsch Gesellsch Chir*. 1895; 24: 240-252.
12. Matanosky GM, Elliott EA. Bladder Cancer Epidemiology. *Epidemiol Rev.* 1981; 3: 203. PMID: 7030761.
13. Rubino G, Coscia GC. (1973) I tumori professionali del tratto urinario. *II Cancro*. 1973; 3:151-159.
14. Rubino G, Scansetti G, Piolatto G, Pira E. The carcinogenic effect of aromatic amines: an epidemiological study on the role of o-toluidine and 4,4′-methylene bis (2-methyl aniline) inducing bladder cancer in man. *Environ Res*. 1982; 27(2):241–254. PMID:7084156.
15. Decarli A, Peto J, Piolatto G, La Vecchia C. Bladder cancer mortality of workers exposed to aromatic amines: analysis of models of carcinogenesis. *Br J Cancer.* 1985; 51(5):707–712. PMID:3994914.
16. Piolatto G, Negri E, La Vecchia C, Pira E, Decarli A, Peto J. Bladder cancer mortality of workers exposed to aromatic amines: an updated analysis. *Br J Cancer.* 1991; 63(3):457–459. PMID: 2003988.
17. Pira E, Piolatto G, Negri E, et al. Bladder Cancer Mortality of Workers Exposed to Aromatic Amines: A 58-Year Follow-up. *J Natl Cancer Inst*. 2010; 102:1096–1099. PMID: 20548022
18. Breslow NE, Day NE. Statistical methods in cancer research. Volume I. The analysis of case-control studies. *IARC Sci Pub*. 1980; (32):5-338. PMID: 7216345.
19. Armitage P. Tests for linear trend in proportions and frequencies. *Biometrics*. 1985; 1:375-386
20. International Agency for Research on Cancer, Global Cancer Observatory, from https://gco.iarc.fr/today/data/factsheets/cancers/30-Bladder-fact-sheet.pdf, last accessed on Feb 1st, 2022.
21. Pira E, Pelucchi C, Piolatto PG, Negri E, Bilei T, La Vecchia C. (2009) Mortality from cancer and other causes in the Balangero cohort of chrysotile asbestos miners. *Occup EnvironMedicine*. 2009; 66(12): 805–809; PMID 19643771.

Table S1. Temporal parameters of the mesothelial tumor cases

Case n. and tumor site	Years of employment in the dyestuff factory	Age at death	Latency period (years)
1-pleura	30	76	51
2-pleura	6	83	64
3-pleura	19	79	45
4-pleura	1	69	33
5-peritoneum	2	58	33
6-peritoneum	6	56	36