

Original

An epidemic of bladder cancer: ten cases of bladder cancer in male Japanese workers exposed to *ortho*-toluidine

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Abstract: Background: *ortho*-Toluidine (OT) was listed as a Group 1 carcinogen by the International Agency for Research on Cancer in 2012 based on epidemiological observations of workers co-exposed to OT and aromatic amines. From 2014 to 2017, several cases of bladder cancer (BCa) secondary to occupational exposure, primarily to OT, were detected in Japan. **Objective:** To describe 10 cases of BCa in male Japanese workers exposed primarily to OT at two plants that produce organic dye and pigment intermediates. **Methods:** Details of the 10 cases were obtained from company records and through a questionnaire and interview. The surrogate level of exposure to each aromatic amine was calculated as the total job-weight/month for each process for each job-year. **Results:** No quantitative exposure data were available. In most cases the surrogate level of exposure to OT was higher than to other amines. All 10 cases were exposed primarily to OT and co-exposed to *para*-toluidine, *ortho*-anisidine, aniline, 2,4-xylydine or *ortho*-chloroaniline. The age range at diagnosis was 41-71 years (mean 56). The duration of OT exposure was 7-28 years (mean 16.5). Disease latency was 16-28 years (mean 21.9). Eight patients were smokers. The main symptom at diagnosis was hematuria (70%). **Conclusions:** The characteristics of BCa cases were associated with a high surrogate level of OT exposure and a disease latency of more than 20 years from the initial OT exposure. The main route of OT exposure was likely through the skin. It is necessary to continue health examinations in these target groups.

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Key words: 2,4-xylydine, Aniline, Bladder cancer, Dermal absorption, *ortho*-toluidine

Introduction

ortho-Toluidine (OT) is a known human carcinogen. Evidence of its carcinogenicity is based on cohort studies of rubber chemical workers and dye workers who developed urinary bladder cancer, and OT was listed as a Group 1 carcinogen by the International Agency for Research on Cancer in 2012^{1,2}. Since there are several routes of absorption for OT, including inhalation, ingestion, and skin contact³, conducting an external exposure assessment to evaluate exposure to OT is complex.

From 2014 to 2015, four male workers and a retiree of a Japanese plant with approximately 40 employees that produces organic dyes and pigment intermediates were diagnosed with bladder cancer (BCa) after having experienced gross hematuria⁴. All five cases were exposed primarily to OT and partially to aniline (AN), 2,4-xylydine (MX), *para*-toluidine (PT), *ortho*-anisidine (OA) or *ortho*-chloroaniline (OCA). An additional four male workers at the same plant and one male worker at another plant (60 employees) belonging to the same company were diagnosed with BCa in 2016 and 2017 after the plants conducted two to four health checkups per year focusing on the detection of urinary tract cancer. In this report, we describe these 10 BCa cases.

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Supplementary materials (Appendices) are available in the online version of this article.

Methods

This study was approved by the Ethics Committee of the School of Medicine of Keio University (approval number: 20160172). Written consent was obtained from all subjects.

Detailed information on job history and health check-ups was provided by the workers' employer, and subjective symptoms and past/current medical histories were obtained by a questionnaire and doctor's interview.

Work description

The plants produce the intermediates of organic dyes and pigments using OT and other aromatic amines as raw materials. Fig. 1 shows a flow diagram of the materials and procedures used by the workers for four OT-related processes: (1) preparation and reaction by mixing OT and diketene in organic solvent; (2) filtering and rinsing the product with organic solvent; (3) drying and packing the product; and (4) distillation of waste organic solvent. Since processes (1) and (4) used closed tanks, they are thought to have resulted in relatively lower OT exposure through the skin and/or respiratory tract, while processes (3) and (4) are thought to have resulted in relatively higher OT exposure.

The workers wore natural rubber gloves up until 2015 at all plants, as well as short-sleeved work clothes (65% polyester and 35% cotton) or cotton shirts in the summer until 2011. The workers used gas respirators for organic solvent work until 2007, and wore masks that protected the wearer from inhaling both organic gas and dust from 2007 to 2016; however, the cartridges of these masks were not routinely changed.

Exposure level estimation

No quantitative OT exposure data were available. Based on the company's records and interviews with long-term employees, extensive data on job-weight/month for each of the four processes and job-years of annual exposure to OT and other aromatic amines (PT, OA, AN, MX or OCA) were collected to estimate the surrogate level of exposure to each aromatic amine. The surrogate level of exposure to each aromatic amine was calculated as the total job-weight/month for each process for each job-year. Job-weight/month was allocated as follows: 0 (none), 1 (1 to 2 days per month), 5 (3 to 9 days per month), or 10 (more than 10 days per month).

The following is an example of how to calculate the surrogate level of exposure to an aromatic amine. Let us suppose that a worker engaged in Processes 1 and 2 at a frequency of 3 to 9 days per month for 10 years, and in Processes 3 and 4 at frequency of more than 10 days per month for 10 years. Thus, we calculate a surrogate level of 5 (job-weight/month) \times 10 years in the process for both

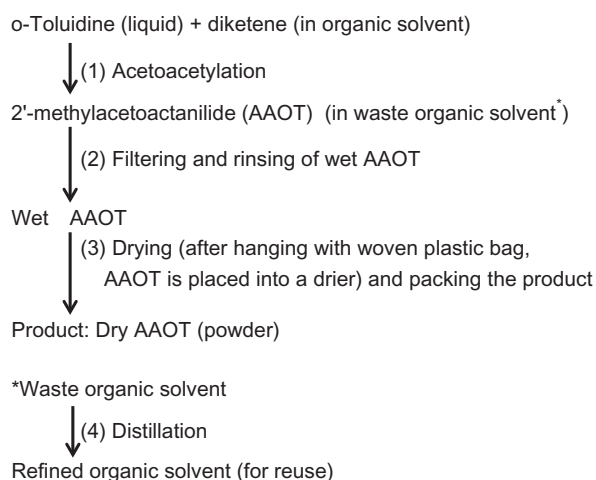


Fig. 1. Flow diagram of the materials and procedures used by the workers

Processes 1 and 2, each of which thus gives a total of 50; and a surrogate level of 10 (job-weight/month) \times 10 years in the process for both Processes 3 and 4, each of which gives a total of 100. We therefore have a surrogate level of 50 + 50 + 100 + 100 = 300.

Results

The surrogate level of exposure to each aromatic amine is shown in Table 1. The workers were exposed primarily to OT (10/10), and co-exposed to AN (9/10), MX (9/10), OA (3/10), OCA (2/10), and PT (1/10). All of the affected workers had been primarily engaged in drying and packing the product made from OT. In most cases, the surrogate level of exposure to OT was higher than exposure to the other amines.

Table 1 shows urinary tract diseases, signs, and symptoms before and at BCa diagnosis. All men are alive in 2017. The age at diagnosis ranged from 41 to 71 years (mean 56 years), and two of the 10 patients were non-smokers. The workers were hired between 1987 and 1997. Duration of exposure to OT, MX, and AN was 7-28 years (mean 16.5 years), 6-24 years (mean 15.0 years), and 3-21 years (mean 13.6 years), respectively. The disease latency from the initial exposure to each chemical was 16-28 years (mean 21.9 years), 18-26 years (mean 22.6 years), and 15-27 years (mean 21.7 years), respectively. The proportion of cases with hematuria, incomplete emptying of the bladder, and dysuria at BCa diagnosis was 70%, 20%, and 20%, respectively. The duration of hematuria before the BCa diagnosis was variable among the workers, ranging from one day to up to one year. Of the 10 cases, four had a history of cystitis, one had interstitial cystitis, and two had ureteral stones.

Table 1. Surrogate exposure levels and urinary tract abnormalities in BCa cases.

Case	1	2	3*	4*	5	6	7*	8	9*	10	
Surrogate level of exposure											
OT	440	395	380	370	310	258	215	160	105	100	
MX	121	315	190	192	155	282	202	80	110	-	
AN	256	122	237	175	142	470	155	107	115	-	
OA	-	-	-	-	-	65	-	-	60	180	
OCA	-	-	-	38	-	-	-	-	75	-	
PT	-	-	-	-	-	-	-	-	-	195	
Smoking status	Pack-years	32	36	0	33	15	39	29.3	10	45	0
Past histories of urinary tract diseases, signs or symptoms: Years before the BCa diagnosis:											
Cystitis	5	11	-	-	21	-	-	11-2 (5 times)	-	-	
Interstitial cystitis	-	-	-	-	-	-	-	-	-	2	
Gross hematuria	0	0	1	1	12, 7, 2	0	-	2	-	0	
Microscopic hematuria	-	-	-	22-1	-	-	-	-	-	-	
Incomplete emptying of the bladder	-	11	-	1-0	21	-	-	2-0	-	2-0	
Dysuria	12, 5	11	17-1	1-0	21	-	-	-	-	0	
Ureteral stones	-	24 [#]	-	22	-	-	-	-	-	-	
Subjective symptom at BCa diagnosis											
Hematuria	+	+	+	+	+	+	-	-	-	+	
Incomplete emptying of the bladder	-	-	-	-	-	-	-	+	-	+	
Dysuria	-	-	+	-	-	-	-	-	-	+	

Cases are ordered based on the surrogate level of OT exposure. The surrogate level of exposure to each aromatic amine was calculated as the total job-weight/month for each process for each job-year. Job-weight/month was allocated as follows: 0 (none), 1 (1-2 days per month), 5 (3-9 days per month), and 10 (more than 10 days per month). OT: *ortho*-toluidine, AN: aniline, MX: 2,4-xylidine, PT: *para*-toluidine, OA: *ortho*-anisidine, OCA: *ortho*-chloroaniline

* Cases that were identified by intensive health checkups conducted by the company.

indicates that symptom occurred prior to employment by the company.

Discussion

This is the first report of an accumulated number of BCa cases due to occupational exposure to OT in Japan. From 2014 to 2017, 10 BCa cases were identified among workers in two plants belonging to a single company that produced intermediates of organic dyes and pigments. Available health and personnel records from the company indicate that one case of BCa was identified prior to 2013.

A causal relationship between occupational exposure to OT and BCa has been reported in several cohort studies performed in the US, UK, and Italy^{1,2}. The study populations had been concurrently exposed to other aromatic amines including 4-chloro-*o*-toluidine, 4-chloro-acetyl-*o*-toluidine³, indigo, 2-mercaptobenzothiazole, phenyl-naphthylamine⁵, AN, or nitrobenzene⁶. The authors concluded that OT was the cause of BCa based on the potent animal carcinogenicity of OT, the large amount of OT used in these plants, and the higher urinary level of OT compared to AN^{6,7}. One study reported a duration of OT exposure of more than 10 years, and disease latency of more than 20 years from initial OT exposure⁶. Based on

these reports, the International Agency for Research on Cancer classified OT as a Group 1 carcinogen in 2012¹.

All of the 10 BCa cases presented here had been exposed to OT. Nine were also exposed to AN and MX, three to OA, two to OCA, and one to PT. In most cases, the surrogate exposure level of OT was higher than that of the other amines. In addition, the mean duration of OT exposure and the disease latency from the initial OT exposure were comparable to those in a published cohort study⁶. Therefore, we concluded that OT was the compound responsible for the 10 BCa cases.

Although there are several routes of absorption for OT, dermal absorption of OT is a particularly important route that should be considered in the prevention of occupational disease⁸. Due to the low vapor pressure of OT at standard ambient temperatures and pressures (20°C, 1 atm)⁹, inhalation of evaporated soluble OT is unlikely to occur without a heating procedure in OT-related processes of filtration, rinsing with organic solvent, and packaging dry product. A cohort study suggested that high external exposure through skin contact may have occurred, since workers in the breathing zone with OT level < 0.5 ppm showed a higher urinary OT level of 1.7 ppm (based

on biological monitoring³⁾. In addition, OT penetrates human skin easily and quickly *in vitro*. OT skin penetration begins within one hour and reaches the maximum penetration rate within 4 to 7 hours¹⁰⁾, and skin absorption is emphasized in the list of occupational exposure limits recommended by the American Conference of Governmental Industrial Hygienists¹¹⁾ and the Deutsche Forschungsgemeinschaft¹²⁾. In this study, the concentration of OT ranged from < 0.1% to 99% in OT-related processes, and workers wore ineffective protective gloves and inappropriate work clothes, which undoubtedly resulted in skin contact with OT. Therefore, we conclude that the main route of OT exposure was through the skin.

Smoking is the most important risk factor for BCa¹³⁾; however, there were two non-smokers among the 10 cases. Three cases were in their 40s and the mean age at diagnosis of BCa was 56 years, which is much younger than the age (more than 60 years) of patients with BCa in the general Japanese population¹⁴⁾. Further investigation is required to determine the causal relationship between OT and BCa in Case 9 because his age at BCa diagnosis (over 70 years) matches the general incidence age, and his surrogate level and duration of OT exposure was lower than almost all of the other cases. Although it is difficult to determine the exact cause (OT or cigarettes) of BCa in Case 9 using the available evidence, it is probable that the patient's occupational exposure to OT caused BCa. Furthermore, the most important factor related to the 10 BCa cases in this study is occupational exposure to OT.

Dermal exposure control and reduction may be required for workers who are at risk of exposure to OT. Therefore, it is necessary to continue to conduct health examinations in these target groups. In addition, disease latency and co-exposure to OT and other aromatic amines (AN and MX) or their products are important considerations.

Conclusions

The characteristics of BCa cases were a high surrogate level of exposure to OT and a disease latency of more than 20 years from the initial OT exposure. The main route of OT exposure was mostly likely through the skin. It is necessary to continue health examinations in these target groups.

Abbreviations

OT: *ortho*-toluidine; BCa: bladder cancer; AN: aniline; PT: *para*-toluidine; OA: *ortho*-anisidine; MX: 2,4-xylydine; OCA: *ortho*-chloroaniline

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Conflicts of interest: None declared.

Supplementary material: This article contains supplementary material (Appendix), which is available in the online version (doi: 10.1539/joh.2017-0220-OA).

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