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New Opportunities for Screening and Early Detection of Bladder Cancer

Thomas J. Mason, PhD[1], William P. Walsh, MA[1], Kathleen Lee, RN[1], and William Vogler, PA[2]

[1] Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111
[2] DuPont Chambers Works, Deepwater, New Jersey 08023

Abstract In the United States, over 51,000 new cases of urinary bladder tumors are diagnosed annually. Approximately 75-85% of all newly diagnosed cases are superficial transitional cell carcinomas (TCCs). Incidence is highest (80% of the cases) in the 50-79 year age group. Recent studies have reported that 21-25% of risk for bladder cancer among United States white males is due to occupational exposure. The DuPont Chambers Works in Deepwater, New Jersey, was a major producer of two chemicals now known to be human bladder carcinogens (β-naphthylamine and benzidine) as well as two suspected human bladder carcinogens [ortho-toluidine and 4,4'-methylene-*bis*,2-chloroaniline (MOCA®)]. Between 1954 and 1982, DuPont screened 1723 exposed employees annually at the Chambers Works using the Papanicolaou test for urinary cytology and microscopic urinalysis. A review of the prior screening program found that employees who developed bladder cancer during this time period were approximately twice as likely to have had hematuria than those comparably exposed who did not develop bladder cancer.

Building on this finding, a three-year screening study evaluated a home self-test for microscopic hematuria to aid early detection of treatable urologic conditions among exposed workers at this chemical plant. Every six months, subjects tested their urine at home for 14 consecutive days, for the presence of blood. A high degree of adherence to our protocol (over 92% completed and returned the self-testing record) as well as high compliance with repeat screening (85% returned for screening in subsequent quarters) demonstrated good acceptance and performance of the recommended schedule of self-testing. Through the first 7 periods of screening, two new cases and one recurrence of TCC of the bladder were detected.

Key words: biomarkers, bladder cancer, early detection, hematuria, screening

In May of 1989, DuPont Chambers Works accepted a research proposal from Fox Chase Cancer Center to develop, execute and analyze a screening program for those in their workforce who had been exposed to known (β-naphthylamine and benzidine) or suspect [4,4'-methylene-*bis*,2-chloroaniline (MOCA®)] bladder carcinogens. Screening for bladder cancer has an extensive history at this particular plant (Table I): from 1929 when the first bladder cancer case was identified among workers exposed to organic dyes, through the early 1930s when cystoscopy was required annually among the workforce at risk for bladder cancer, to 1954 when urinary cytology replaced cystoscopy as the primary screening test [1–22].

Prior to the adoption of our protocol, workers at potential risk for occupational bladder cancer at this plant had annual microscopic analyses of their urine for the presence of red blood cells, as well as urinary cytology. We proposed quarterly microscopic analysis of urine specimens, semi-annual cytologies, and semi-annual self-testing for hematuria lasting 14 consecutive days. Self-testing and cytologies were scheduled in alternating quarters; microscopic analysis was done each quarter. Figure 1 presents the protocol followed.

With the publication of Guirguis *et al.* [18] on the detection of autocrine motility factor (AMF) as a marker for bladder cancer, our then-draft proposal to DuPont was expanded to include baseline AMF assays, and to include AMF determinations as part of the diagnostic workup for those who test positive. This schedule has been followed since the commencement of our program. Urine specimens on all participants are banked and will be used to assess the utility of

TABLE I. Chronology of Events Which Influenced Screening for Bladder Cancer at the DuPont Chambers Works

1919	Production of β-naphthylamine began.
1929	First bladder cancer case identified at DuPont Chambers Works.
1931	3 new cases identified. Cystoscopic exam required as part of routine physical examination.
1932	12 new cases identified.
1933	Corporate acceptance of responsibility for all medical and surgical bills as well as any resultant disability started at this time.
1934	J Ind Hyg Toxicol WC Hueper. Cancer of the urinary bladder in workers of chemical dye factories and dyeing establishments: A review.
1934	J Urol GH Gehrmann recognized possible association with β-naphthylamine and benzidine, and described industrial hygiene practices, which resulted in a marked reduction in exposure to β-naphthylamine.
1936	JAMA GH Gehrmann reported on the time-dependent characteristics of 24 cases of carcinoma of the bladder and 39 cases of papilloma. Discussed preventive measures, plant operative and medical, which were predicted to eliminate the incidence of these tumors.
1937	J Urol EE Evans, HD Wolfe, DM Gay, VD Washburn, RS Ferguson report on causative agents, routine cystoscopic examination as a control measure, pathology, treatment and the clinical significance on 83 cases of bladder tumors.
1938	Arch Pathol WC Hueper A General Review: "Anilin tumors" of the bladder.
1948	Proc IX Intl Congr Ind Med GH Gehrmann presented the findings of 15 years of clinical and experimental research of the problem of bladder tumors among workers of the DuPont Chambers Works.
1954	Urinary cytology replaces cystoscopy as primary screening test.
1956	Production and handling of β-naphthylamine discontinued.
1962	4,4′-methylene-*bis*,2-chloroaniline (MOCA®) manufacturing begun, continuing until 1979.
1967	Benzidine production discontinued. Use in manufacturing continued through 1972.
1977	State-of-the-Art Conference on Bladder Cancer Screening sponsored by the U.S. National Cancer Institute (NCI). DuPont Medical presents findings from the cytology screening program which started in 1954.
1979	NCI negotiates a collaborative research agreement with DuPont.
1984	Conference on Medical Screening and Biological Monitoring for the Effects of Exposure in the Workplace. NCI and DuPont present an update on the ongoing cytology program of the Chambers Works. Between 1954 and December 1982, 142 developed bladder cancer, 1581 did not.
1987	J Urol IM Thompson found 4% of 2005 men over age 40 had asymptomatic microhematuria, 22% of whom had significant urologic disease. J Urol EM Messing *et al.* reported on the screening characteristics of a reagent strip for the presence of blood in the urine in comparison to the usual microscopic urinalysis.
1988	J Natl Cancer Inst R Guirguis *et al.* detected an Autocrine Motility Factor (AMF), a cytokine which stimulates motility in human tumor cells, in urine of patients with bladder TCC. Am J Ind Med E Ward reports on 2 bladder tumors found in men under age 30 exposed to MOCA®.
May 1989	Agreement between DuPont Chambers Works and Fox Chase Cancer Center.
Sept 1989	Screening protocol presented at NIOSH-sponsored International Conference on Bladder Cancer Screening in High-Risk Groups.
Nov 1989	FCCC screening program begun at Chambers Works with employees who worked with MOCA®.
Dec 1989	J Urol EM Messing found 19% of 235 men age 50 and over had hematuria detected

TABLE I *(Continued)*

	by weekly dipstick testing. Fifteen of these 44 patients (34%) had significant urologic disease.
Dec 1989	NIOSH releases preliminary report on 14 cases of bladder cancer among Goodyear Tire and Rubber Company workers. o-toluidine is said to be the "most likely cause."
May 1990	o-toluidine-exposed workers invited to participate in screening program.

AMF as well as other assays, most notably epidermal growth factor, tumor cell collagenase stimulating factor, and p53 as markers for bladder cancer. Guirguis has patented a specimen collection and concentration system (CDI Shuttle System™) which is being utilized in our new studies (Fig. 2).

Each Cyto-Shuttle contains a specially laminated 25-mm membrane enclosed in a two-piece plastic housing and comes with a debris-filtering Shuttle device. Used together, these devices form a proprietary dual filtration system that removes debris and collects cells onto the membrane inside the Cyto-Shuttle. The LC-Shuttle is the only flow-rate independent batch chromatography system, *i.e.*, one that allows the resins to mix freely with the clinical sample. This eliminates operator preparation variability and ensures reproducible results.

During the planning and development phase of our protocol, we initiated an information campaign at the plant which utilized articles in the plant union and community newspapers, as well as electronic mail messages which were transmitted plant-wide. We met with and addressed the concerns of the Chemical Workers Association, many of whose officers were production line workers early in their careers and are now participants in the screening program.

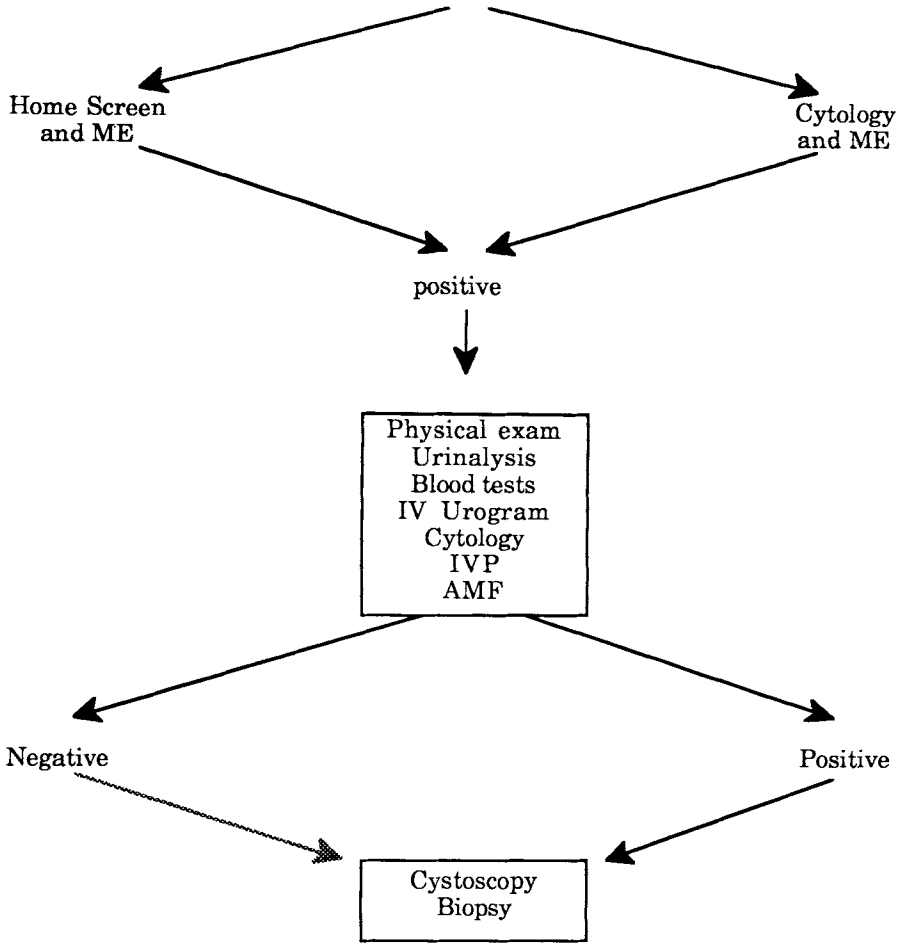
Once the protocol was finalized, a number of worksite information meetings targeted small groups of workers in convenient work areas. The high participation rate among these workers is partly due to the effort which was expended during the recruitment phase. Workers exposed to MOCA® were recruited first. Although MOCA® is classed as a potential human carcinogen, workers were made aware of renewed concerns about exposure to this chemical.

Table II presents the number of individuals recruited into the At-Home Screening Program by quarter. The large number of individuals recruited during the third quarter reflects an influx of workers exposed to ortho-toluidine following the release of a preliminary NIOSH report on cases of bladder cancer among Goodyear Tire and Rubber workers exposed to this chemical.

TABLE II. Accrual by Quarter

Quarter	Number Accrued
1	764
2	31
3	93
4	23
5	26
6	2
7	0
Total Number Accrued:	**939**

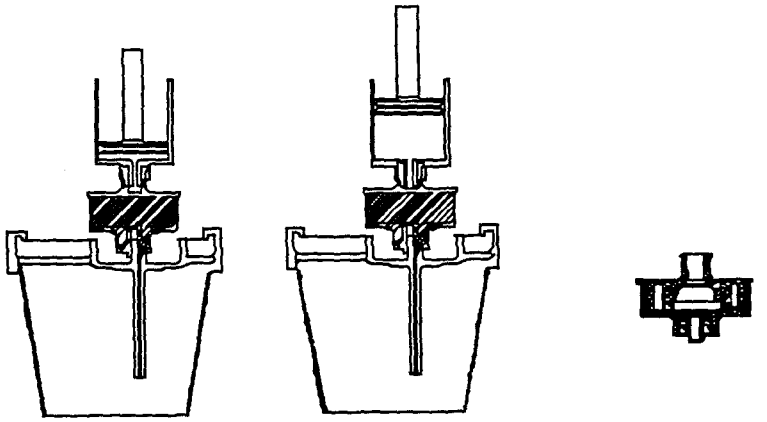
Table III presents a summary of our screening activities at the DuPont Chambers Works through the first 7 quarters. As evidenced by the return compliance of those participating in our protocol, workers have readily accepted our recommendations. We predicted that 15% of the participants would test positive during the odd-numbered quarters which require self-testing for 14 consecutive days. This held for quarters 1 and 3, and has subsequently diminished. In the even-numbered quarters, in which the participants provide a single urine specimen for microscopic analysis as well as cytology, the number of individuals with abnormal tests is considerably smaller than the number who are found with multi-day testing. The last column of Table III further refines these numbers to provide an assessment of numbers and percents of indi-



ME	microscopic analysis
IVP	intravenous pyelogram
AMF	autocrine motility factor
Urinalysis	routine analysis, culture and sensitivity
Blood tests	complete blood count, serum creatinine, blood urea nitrogen, prothrombin time, partial thromboplastin time, glucose

Alternating
Every 3 mos.

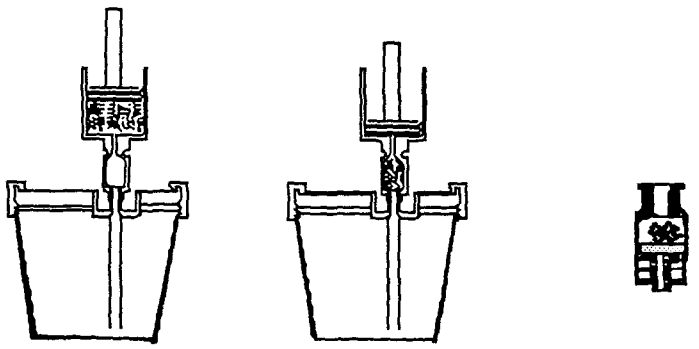
Fig. 1. Protocol for bladder cancer screening.



Attach Cyto-Shuttle to Collection Cap

Draw Sample into syringe through Cyto-Shuttle

Detach Cyto-Shuttle and Ship to the lab



Draw Sample into syringe through LC-Shuttle

Empty syringe through the LC-Shuttle

Detach LC-Shuttle and Ship to the lab

Fig. 2. CDI Shuttle System™.

TABLE III. Participation and Screening Test Results by Quarter Among DuPont Chambers Works Employees

Quarter	No. Screened	Return Compliance (%)	No. Abnormal	No. With ABN Hx	No. Screened With NL Hx	Abnormal for First Time
1	764	--	116 (15.2%)	0	764	116 (15.2%)
2	688	86	31 (4.5%)	98	590	14 (2.4%)
3	798	89	126 (15.8%)	112	686	55 (8.0%)
4	771	85	56 (7.3%)	164	607	12 (2.0%)
5	810	87	109 (13.5%)	180	630	30 (4.8%)
6	772	84	55 (7.1%)	194	578	14 (2.4%)
7	794	86	95 (12.0%)	211	583	23 (3.9%)
						264 (28.1%) (% of Total No.)

Return Compliance = Persons recruited in prior quarters who return to be tested in subsequent quarters.
Abnormal = Microscopic examination: 3 or more red blood cells/high power field; Hemastix®: trace amount or greater of red blood cells, hemoglobin or myoglobin detected; Cytology: Class III, markedly atypical transitional cells, suspicious but not diagnostic for malignancy, or Class IV, cells highly suspicious for malignancy, or Class V, cells positive for malignancy.
ABN Hx = Persons who have had abnormal test results on our protocol in any prior quarter.
NL Hx = Persons who have never had abnormal test results on our protocol in any prior quarter.

TABLE IV. Results of Urologic Evaluation—Subjects With Hematuria and/or Abnormal Cytology

Diagnoses of NEW Conditions	Number
Transitional Cell Carcinoma	2
Renal Calculi	3
Ureteral Calculi	1
Prostatitis	5
Bladder Outlet Obstruction/Stricture	4
Benign Prostatic Hypertrophy	13
Urinary Tract Infection	5
Testicular Edema Due to Trauma	1
Cystitis	16
Bladder Dysplasia	2
Chronic Myelocytic Leukemia	1
Medullary Sponge Kidney	1
Mass in Epididymis	1
Prostatic Urethritis	1
Bladder Irritation Due to Crystals	1
Renal Cyst	1
Unknown Etiology	32
TOTAL	**90**

TABLE V. Bladder Tumor Case A

◆ 59 year old college-educated white male manager

Risks: —Began at DuPont in 1954; had exposure to benzidine
—Non-smoker; has never used any tobacco product
—Cup-a-day coffee drinker; has lived in Wilmington for 36 years

Sx: Microscopic urinalysis: 2–4 RBC/HPF
Hemastix® testing: hematuria on 9 of 14 days (trace to 1+)
Cytology: Class I (no evidence of dysplasia)
No other signs or symptoms

Dx:
1. Grade II superficial transitional cell carcinoma (TCC) of the bladder
2. Benign adenomatous hyperplasia of the prostate with focal prostatic intraepithelial neoplasia

Tx: **April 1990**, Transurethral Resection of Bladder Tumor

Status: January 1992, NED by cystoscopy

TABLE VI. Bladder Tumor Case B

	♦ 49 year old high-school educated white male chemical operator
Risks:	—Began at DuPont in 1973; had exposure to **α-naphthylamine only** —1/2 pack-a-day cigarette smoker —4 cups-a-week coffee drinker; life-long So. New Jersey resident —Previous exposure while working as a hair-dresser
Hx:	March 1985, Resection of Grade II papillary TCC of the bladder January 1990, Transurethral prostatectomy for BPH
Sx:	August 1990, Microscopic u/a: no RBCs seen Cytology: Class 0 (acellular) Class III (atypical transitional cells) No other signs or symptoms
Dx:	Severe dysplasia with foci of carcinoma *in situ*
Tx:	**Sept 1990**, Transurethral Resection of Bladder Tumor 1990–1991, Intravesical Bacillus Calmette-Guerin therapy
Status:	March 1992, NED by cystoscopy

TABLE VII. Bladder Tumor Case C

	♦ 60 year old high-school educated white male pipefitter
Risks:	—Began working at DuPont in 1974 —Had exposure to o-toluidine (6000 hours), claims benzidine —Former smoker; smoked 25 cigarettes a day for 34 years —20 cups-a-week coffee drinker; lived in S.E. Penna. for 30 years
Sx:	May 1991, Microscopic u/a: no RBCs seen Hemastix® testing: hematuria on 8 of 13 days (trace to 3+) Cytology: Class I (no evidence of dysplasia) Aug & Nov 1991, Feb 1992: All Screening Negative!
Dx:	Grade II papillary TCC of the bladder, lamina propria and muscular invasion absent
Tx:	**March 1992**, Transurethral Resection of Bladder Tumor

viduals who test positive for the first time in a given quarter. These data will be used to determine how many periods of self-testing for microhematuria are required to identify individuals with persistent hematuria.

Table IV provides a distribution of individuals identified with abnormal screening tests for whom new conditions were diagnosed. Diagnostic evaluation of all persons who have had abnormal test findings has not been completed; however, our preliminary findings are consistent with the available literature. Referring to Thompson's earlier paper [16], the "serious but treatable" conditions identified at the DuPont Chambers Works were found in a comparable percentage of this workforce. These conditions include bladder cancer, urinary calculi, urethral stricture, chronic pyelonephritis, polycystic kidney disease, and mycobacterial cystitis.

Characteristics of the bladder cancer cases

identified as a result of our screening program are presented in chronological sequence (Tables V, VI, VII). Case A (Table V) was positive after Hemastix® testing for 9 of the 14 days; microscopic urinalysis was positive [2–4 red blood cells/high power field (RBC/HPF)] and cytology was Class I. Transurethral resection was performed in April 1990, and as of January 1992 there was no evidence of disease (NED) by cystoscopy.

Case B (Table VI) had a prior history of bladder cancer. A negative cystoscopy was recorded three months before discovery of Class III cytology. The participant's prior history influenced the urologist to perform random biopsies, even though the cystoscopy had not identified a specific lesion. As of March 1992, this participant is disease free.

Case C (Table VII) tested positive by Hemastix® on 8 of 13 days, but delayed diagnostic evaluation for almost a year. During this period, continued screening tests were negative. Finally, after reminders from our nursing staff, he agreed to accept our recommendation for diagnostic evaluation and a papillary transitional cell carcinoma (TCC) of the bladder was identified.

FUTURE DIRECTIONS

Given the number of new cases of bladder cancer identified each year in the United States, and estimates that approximately 25% of those are attributable to occupation [23,24], we are concentrating our efforts on identifying and enrolling persons at risk for occupationally related bladder cancer into similar screening programs. Our experience to date suggests that multi-day testing is appropriate and readily acceptable.

These modified protocols provide an opportunity to evaluate recent improvements in biologic specimen collection and banking procedures. The CDI Shuttle System™, which was developed and patented by Guirguis, will be evaluated. This device facilitates evaluating the utility of a number of assays (autocrine motility factor, epidermal growth factor and receptor, tumor-cell collagenase stimulating factor, and p53 protein) in the identification and medical management of bladder cancer.

Our present activities at the DuPont Chambers Works will continue through May 1993, when recommendations will be made for subsequent screening activities. For purposes of chemoprevention, serious consideration should be given to using screening programs as efficient platforms for fielding chemoprevention trials which incorporate biomarkers in the identification of persons at increased risk for bladder cancer as well as predict their likelihood of experiencing recurrent disease.

REFERENCES

1. Hueper WC: Cancer of the urinary bladder in workers of chemical dye factories and dyeing establishments: A review. J Ind Hyg Toxicol 16:255–281, 1934.
2. Ferguson RS: Symposium on anilin tumors of the bladder: Introduction. J Urol 31:121–126, 1934.
3. Gehrmann GH: Symposium on anilin tumors of the bladder: The carcinogenetic agent—chemistry and industrial aspects. J Urol 31:126–137, 1934.
4. Gay DM: Symposium on anilin tumors of the bladder: Pathology of anilin tumors of the bladder. J Urol 31:137–148, 1934.
5. Anderson LW: Symposium on anilin tumors of the bladder: Incidence, symptoms and signs, results of survey. J Urol 31:148–154, 1934.
6. Washburn VD: Symposium on anilin tumors of the bladder: Treatment of anilin tumors of the urinary bladder. J Urol 31:155–173, 1934.
7. Gehrmann GH: Papilloma and carcinoma of the bladder in dye workers. JAMA 107(18):1436–1439, 1936.
8. Evans EE: Causative agents and protective measures in the anilin tumor of the bladder. J Urol 38:212–215, 1937.
9. Wolfe HD: Routine cystoscopic examination as a control measure in anilin tumor of the bladder. J Urol 38:216–220, 1937.
10. Gay DM: Pathology of anilin tumors of the bladder. J Urol 38:221–231, 1937.
11. Washburn VD: The treatment of the anilin tumors of the urinary bladder. J Urol 38:232–242, 1937.
12. Ferguson RS: Clinical significance of the anilin tumor of the bladder. J Urol 38:243–250, 1937.
13. Hueper WC: General review: "Anilin tumors" of the bladder. Arch Pathol 25:858–899, 1938.
14. Gehrmann GH, Foulger JH, Fleming AJ: Occupational carcinoma of the bladder. Proc IX Intl Congr Ind Med 472–475, 1948.
15. Mason TJ, Prorok PC, Neeld WE Jr, Vogler WJ: Screening for bladder cancer at the DuPont Chambers Works: Initial findings. J Occup Med 28(10): 1011–1016, 1986.
16. Thompson IM: The evaluation of microscopic hematuria: A population-based study. J Urol 138: 1189–1190, 1987.

17. Messing EM, Young TB, Hunt VB, Emsto SE: The significance of asymptomatic microhematuria in men 50 or more years old: Findings of a home screening study using urinary dipsticks. J Urol 137:919–922, 1987.
18. Guirguis R, Schiffman E, Liu B, Birkbeck D, Engel J, Liotta L: Detection of autocrine motility factor in urine as a marker of bladder cancer. J Natl Cancer Inst 80:1203–1211, 1988.
19. Mason TJ, Vogler WJ: Bladder cancer screening at the DuPont Chambers Works: A new initiative. J Occup Med 32(9):874–877, 1989.
20. Guirguis R, Javadpour N, Shararch S, Biswas C, El-Amin W, Mansur I, Kim JS: A new method for evaluation of urinary autocrine motility factor and tumor cell collagenase stimulating factor as markers for urinary tract cancers. J Occup Med 32(9): 846–853, 1989.
21. Ward EW, Halperin W, Thun M, Grossman HB, Fink B, Koss L, Osorio AM, Schulte P: Bladder tumors in the two young males occupationally exposed to MBOCA. Am J Ind Med 14:267–272, 1988.
22. Messing EM, Young TB, Hunt VB, Wehbie JM, Rust P: Urinary tract cancers found by home screening with hematuria dipsticks in healthy men over 50 years of age. Cancer 64:2361–2367, 1989.
23. Silverman DT, Levin LI, Hoover RN, Hartge P: Occupational risks of bladder cancer in the United States: I. White men. J Natl Cancer Inst 81:1472–1480, 1989.
24. Silverman DT, Levin LI, Hoover RN: Occupational risks of bladder cancer in the United States: II. Nonwhite men. J Natl Cancer Inst 81:1480–1483, 1989.