Bladder cancer and occupational exposure to metalworking fluid mist: a counter-matched case–control study in French steel-producing factories

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Bladder cancer and occupational exposure to metalworking fluid mist: a counter-matched case-control study in French steel-producing factories.

Régis Colin[1], Michel Grzebyk[1], Pascal Wild[2], Guy Hédelin[1], Eve Bourgkard[1*]

* Correspondence to Eve Bourgkard, Department of Occupational Epidemiology, Occupational health and safety Institute (INRS), 1, rue du Morvan - CS 60027 - 54519 Vandœuvre -les-Nancy, France (e-mail: eve.bourgkard@inrs.fr)

Authors affiliations:

[1]Department of Occupational Epidemiology, Occupational health and safety Institute (INRS), Vandœuvre-les-Nancy, France

[2]Department of Scientific Management, Occupational health and safety Institute (INRS), Vandœuvre-les-Nancy, France

KEYWORDS: bladder cancer; metalworking fluids; counter-matching.

ABSTRACT:

Objectives

To assess the relationship between occupational exposure to metalworking fluids (MWFs) in the steel-producing industry and bladder cancer incidence.

Methods

A nested case-control study on bladder cancer was set up in a cohort of workers from six French steel-producing factories. Three controls were randomly selected for each incident bladder cancer case diagnosed from 2006-2012. Controls were matched to cases on age at diagnosis and counter-matched on a surrogate measure of exposure to MWFs derived from a job-exposure matrix. Cases (n=84) and controls (n=251) were face-to-face interviewed. Experts assessed occupational exposure to MWFs (straight, soluble, synthetic) using questionnaires and reports from factory visits. Occupational exposures were based on three metrics: duration, frequency-weighted duration, cumulative exposure index. Conditional multiple logistic regressions were used to determine Odds-Ratios and Confidence Intervals (CI95%), taking non-occupational and occupational exposure into account.

Results

In the 25 years before diagnosis, ORs increased significantly with duration of exposure to straight MWFs (OR=1.13 [1.02-1.25]) and increased with frequency-weighted duration of exposure to straight MWFs (OR=1.44 [0.97-2.14]). These results remained valid after adjusting for duration of smoking, average number of cigarettes smoked per day, time since smoking cessation and exposure to polycyclic aromatic hydrocarbons (PAH). ORs also increased with soluble MWFs, but not significantly. No significant association was found with older exposures to MWFs or with exposure to synthetic MWFs.

Conclusion

Our findings may be explained by the presence of carcinogens (such as PAH) in mineral oils principal component of straight oils. Prevention therefore remains necessary in sectors using MWFs.

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WHAT THIS PAPER ADDS?

1. **What is already known about this subject?**

   Historically, metalworking fluids (MWFs) have been linked to increased incidence of bladder cancer. However, changes to composition and practices call for an updated study.

2. **What are the new findings?**

   This counter-matched case-control study aimed at assessing the relationship between occupational exposure to MWFs in the steel-producing industry and bladder cancer risk. Occupational exposures to straight MWFs in the last 25 years appear to have an effect on bladder cancer incidence. Our study cannot exclude a relationship with exposure to soluble MWFs and does not detect any relationship with synthetic MWFs.

3. **How might it impact on policy or clinical practice in the foreseeable future?**

   Our findings may be explained by the presence of carcinogens (such as PAH) in mineral oils principal component of straight oils. Prevention therefore remains necessary in sectors using MWFs.

ORIGINAL ARTICLE:

Metalworking fluids (MWFs) are used during metal working processes, electroerosion, metal deformation, molding operations to lubricate, cool and remove debris from the surface of the workpiece. MWFs can be classified based on their composition: (i) straight MWFs (mineral oil, no water); aqueous MWFs including (ii) soluble MWFs (mineral oil emulsified in water), and (iii) synthetic MWFs (water with soluble compounds, no mineral oil). MWFs contain additives, such as lubricants, extreme-pressure resistant, antimisting, antiwear, and coloring agents, corrosion inhibitors, biocides, biostatics or perfumes [1]. When using MWFs, aerosols of cutting fluids, known as oil mist, are generated. These aerosols may remain airborne for several hours, and are often present in workers' breathing zones. The tiny, floating droplets making up these aerosols contain both the basic ingredients of MWFs and derivatives produced by thermal degradation. In addition, they can contain machined materials, or microorganisms when using aqueous fluids [1]. Among these elements, some known or suspected carcinogens have been identified, such as polycyclic aromatic hydrocarbons (PAHs) and nitrosamines [2].

In 1984, according to the International Agency of Research on Cancer (IARC), "there [was] sufficient evidence from studies in humans that mineral oils (containing various additives and impurities) that have been used in occupations such as mulespinning, metal machining and jute processing are carcinogenic to humans". Exposure in these occupations "[has] been associated strongly and consistently with the occurrence of squamous-cell cancers of the skin, and especially of the scrotum" [3, 4]. In the literature, an increased risk of bladder cancer has been reported for machinists and mechanics, both personnel categories using MWFs [3, 4]. Most of these studies relate to relatively old (prior to the mid-1990s)

employment periods. However, more recent follow-up and exposure studies also reported an increased risk of bladder cancer among workers exposed to oil mist [5-9]. The composition and nature of MWFs have changed in recent decades, with reductions in the PAH content of straight MWFs from the mid-1980s [10]. However, chemical analyses continue to show PAH enrichment or significant concentrations of nitrosamines in MWF aerosols [11]. Since the last quarter of the 20th century, aqueous MWFs were increasingly used, representing 37% of MWFs used in France in 1970 compared to 49% in 2009. Simultaneously, the proportion of straight MWFs sold decreased from 63% to 51% [12, 13]. Current data do not allow us to determine whether these changes contributed to a decreased incidence of bladder cancer [14, 15].

In a previous study of mortality in French steel-producing factories, we assessed occupational exposure based on a factory-specific job-exposure matrix (JEM)[5]. Secondary results indicated excess mortality from bladder cancer for workers exposed to oil mist (RR=2.44, 95%CI=1.06-5.60) [5].

Based on these findings, it appears necessary to identify and quantify the risk of bladder cancer associated with the different types of MWFs to which workers may be exposed, particularly since the mid-80s.

In this paper, we report the findings of a nested case-control study involving a cohort of workers from six French steel-producing factories. The study was designed to assess possible relationships between occupational exposure to the three types of MWFs and the risk of bladder cancer, taking occupational and non-occupational factors into account.

MATERIALS AND METHODS

- **Study population**

The cohort included 22,795 male workers from six French steel-producing factories, employed for at least 1 year in the same factory, between January 1960 and June 1997. Data from two of the six factories (initial cohort 1) were gathered during our previous study [5]. For the remaining four factories (initial cohort 2), cohort members were identified based on payroll records. The cohort included workers from the whole steel-manufacturing chain (coke oven, sintering, blast furnace, steel mill, sheet mill, cold rolling mill, hot rolling mill, and pipe mill), as well as support departments (materials and supplies, maintenance, transport, research, administration). Incident cases and controls were selected from this cohort. All confidentiality, safety and security procedures were approved by the French legal authorities.

- **Cohort-based estimation of MWF exposure: a surrogate for MWF exposure**

In our previous study [5], a JEM including semi-quantitative assessment of exposure to MWFs was set up for two of the six factories. The same JEM was used for the four other factories to assess the correspondence between periods of work at the four factories and MWF exposure. The pipe and cold rolling mills were not included in the JEM, but the corresponding job titles were related to the most relevant MWFs job-exposure periods.

For each subject in the cohort, a surrogate cumulative exposure index was calculated, by summing the product of duration and intensity of exposure for each job, considering a ten-year lag [6] from the age at bladder cancer diagnosis expected according to French incidence rates (68 years). Four MWF-exposure strata were defined based on the distribution of the surrogate cumulative index for all cohort members. Since a large number of subjects were not exposed to MWF, level zero was considered as the “non-exposed” class. The three other classes were defined by the tertiles of the non-zero surrogate cumulative index.

- **Case ascertainment**

Cases were defined as any men in the cohort diagnosed with primary bladder tumor (both invasive tumors and in situ carcinomas) during the 2006-2012 period. Follow-up was retrospective before 2010, and prospective from 2010. Cancer cases were classified based on the WHO International Classification of Diseases for Oncology, Third Edition (ICD-O-3). Cases were identified by matching the identity data of the cohort subjects, using ICD-codes (C670 to C679), with two sources of information: (i) the clinical information databases from all the hospitals and clinics within a 50-km radius of the factories; and (ii) the national records of requests for exemption from medical expenses through which the French health care system waives payment for treatment for patients with long-term diseases, including cancers. Date of cancer diagnosis, topography, morphology, cancer staging and cell grade were obtained from patients' medical files to verify that the diagnosis of bladder cancer had been histologically validated. To be included in this study, cases had to be alive on the date of interview.

- **Control selection**

For each case, a risk set was set up consisting in all cohort members at risk at the age of diagnosis of the case whatever the date and belonging to the same initial cohort than the case (online figure 1).

Within each risk set, and for each subject, a surrogate cumulative exposure index corresponding to the sum of the product of exposure duration and intensity for each job, up to a reference date, with a 10-year lag, was calculated using the surrogate estimation of MWF exposure. The reference date corresponded to the date of diagnosis for cases and to

the date on which matched controls reached the age of the case at diagnosis. All workers of the risk set were assigned to one of the four surrogate exposure strata previously defined (online figure 1).

Three controls, alive at the date of data collection, were randomly selected from the risk set, counter-matched on the MWF surrogate exposure strata [16, 17] (online figure 1). This counter-matching involves selection of three controls who have a different occupational surrogate exposure level from the case and from one another at the age of the case at diagnosis [18]. Thus, each of the three controls and the case belonged to a different stratum. The sampling weights used for the statistical analysis of these data are the stratum-wise numbers of the risk set members, whether alive or not at the date of data collection.

- **Data collection**

Cases and controls received a letter explaining the objective of the study, the modalities of participation, and information for an appointment. Participants signed written informed consent forms before the interview was conducted.

Participants were interviewed face-to-face at home by trained staff using a series of questionnaires. One questionnaire covered socio-demographic characteristics and medical histories, in particular regarding chronic bladder diseases, personal or family history of cancer, chronic bladder irritations (urinary schistosomiasis or urolithiasis), and abdominopelvic radiography.

Non-occupational parameters were also recorded: smoking and drinking habits, and fruit and vegetable consumption. Environmental arsenic exposure in drinking-water was determined from residential history.

A full occupational history was gathered by collecting detailed information on the jobs held for at least 1 month throughout the subject's career. For each job, the main tasks performed and the company's activity were described. A questionnaire relating to 43 tasks potentially involving exposure to bladder carcinogens (aromatic amines, PAHs) was used [19].

Moreover, for each job within the 17 steel-producing or maintenance sectors identified, a sector-specific questionnaire focused on the activities performed. Thus, detailed information was collected about tasks exposing to atmospheric PAH, exposure to solvents (type and conditions of use), exposure to MWFs - including nature and appearance of the fluids (oily, milky or transparent) - tasks performed, use of personal protective equipment, use of collective protective equipment, and type of machining-tools used.

During on-site visits, information was collected on exposure for present and past processes, working conditions and ventilation systems, and the MWFs used (as determined from the safety data sheets in each workshop for each factory for the 1980-2010 period).

- **Assessing occupational exposure in cases and control**

Present and past occupational exposures were coded by two experts (one senior occupational hygienist and one senior occupational epidemiologist) with a thorough understanding of the processes and history of each facility. For each subject, exposure was assessed for all jobs occupied (job title, workplace, start date, end date) during his career. Experts were blind to case and control status when gathering this data from the available occupational information: responses to occupational questionnaires, site visit reports, and the list of fluids and oils used in each workshop.

The following occupational exposures were coded: MWFs, PAHs except MWFs, chlorinated solvents, nitrosamines, and diesel exhaust fumes. Depending on occupational exposure

levels, up to four exposure indicators were coded: presence, intensity, nature and frequency of exposure. An exposure intensity code on a 0-1-2 scale was defined for MWFs, and chlorinated solvents, and on a 0-1-2-3 scale for PAHs. For nitrosamines and diesel exhaust fumes, only the presence of exposure could be recorded. To assess the intensity of exposure to MWFs, experts identified several typical jobs associated with different intensity levels (online table 1). These typical jobs were used as coding benchmarks. For PAHs, the correspondence between job period and intensity code in the JEM from our previous study [5] was used as a reference. Moreover, intensity levels were confirmed by the experts based on responses to the occupational questionnaires, and were adjusted if personal or collective protective equipment was used. Information on exposure to chlorinated solvents and diesel exhaust fumes was collected during the interview.

The nature of MWFs (straight oils, soluble oils, and synthetic fluids) was obtained from multiple sources: questionnaires, workshop visits and safety data sheets.

The exposure frequency was coded as <10%, 10-50% and >50% based on the proportion of working time during which workers were exposed.

Occupational exposure was explored using three different metrics: duration of exposure at an intensity level $\geq$ 1, frequency-weighted duration of exposure (sum of duration x frequency), and cumulative exposure index (sum of duration x frequency x intensity).

For each case and his controls, exposure was calculated up to a reference date. For cases, the reference date was the date of diagnosis; for controls the reference date was matched to the age of the case at diagnosis.

- **Smoking habits**

Smokers were defined as those who had smoked more than 100 cigarettes in their lifetime and former smokers those who had quit smoking at least one year previously. The others were classed as never-smokers. Details on lifelong smoking habits were collected during the interview. Smoking was assessed based on duration of smoking, pack-years, mean number of cigarettes smoked per day and time since cessation, and summarized as a smoking status (never, former, current smoker).

- **Statistical analysis**

The association between risk of bladder cancer and various exposure scenarios was assessed by conditional multiple logistic regression, estimating Odds-ratios (ORs) and 95% bilateral confidence intervals (95% CIs). To account for the counter-matched design, the logarithm of the number of subjects in the surrogate exposure strata of the risk set was incorporated as an offset when fitting the model [20].

First, to identify confounders, each non-occupational exposure index was included separately. Second, each occupational exposure index was included separately, adjusting for selected confounders (smoking duration, average cigarettes smoked per day and time since smoking cessation). Third, all occupational exposures were considered together in multiple conditional logistic regression models adjusting for the three smoking metrics. The models included the 3 types of MWFs as well as the other occupational exposures for which a *P*-value of less than 0.20 was obtained in the previous analysis. Three multiple analyses were considered, first based on duration of exposure (in year), second on frequency-weighted duration (in full-time equivalent year), and finally on cumulative exposure index (in intensity.year).

While the selection process of the controls was based on a surrogate exposure which was lagged 10 years, the exposure metrics used in the statistical analyses were lagged 5 years. These exposure metrics further considered two time windows: 5-25 years and 25+ years before the reference date.

Sensitivity analysis was performed by comparing different combinations of lag-duration (≥ 5 years, in accordance with the empirical induction period for bladder cancer [21-23]) and exposure period using the Akaike information criterion (online figure 2). Similarly to the exposure metrics, the smoking variables were also lagged 5 years.

The threshold for significance was set at $P<0.05$. Our hypothesis was that occupational exposure effects were deleterious (OR > 1), we therefore used one-tailed tests [24, 25]. All statistical analyses were performed using STATA software (StataCorp. 2014. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

RESULTS

- **Study population**

Of the 157 eligible cases, 85 (54%) were interviewed. Reasons for non-participation were death (33%), not interested (32%), health condition (13%) and unreachable (22%). For 83 of the cases interviewed, three controls per case were also interviewed. For one case, only two controls were included because no control from the third strata agreed to participate in the study. Another case was excluded from the study because of a lack of appropriate controls. Given that a single control could match several cases, the control population included 200 workers, but the overall study included 84 cases and 251 controls. The median age at diagnosis was 63.4 years.

Among eligible cases, those who declined to participate were on average born earlier (age: 68.7 (SD, 9.0) in 2010) than those who participated (65.8 (SD, 7.5) years-old in 2010) ($P<0.05$). Although participating cases spent longer working in the factories (18.2 years (SD, 8.3) than cases who did not participate (14.5 years (SD, 8.5)), no statistically significant difference was found between the two groups in terms of cumulative occupational surrogate exposure to MWFs.

- **Non-occupational characteristics of participants**

Characteristics of the cases and the controls are shown in table 1, as well as unadjusted ORs. Unadjusted ORs increased with the duration of smoking ($p < 0.001$), the mean number of cigarettes smoked per day ($p=0.002$) and pack-years ($p < 0.001$) and declined with time since smoking cessation ($p=0.002$). Only two cases and two controls were exposed to environmental arsenic (data not shown). No statistically significant difference between cases and controls was observed for educational category, diet, family history of bladder cancer, personal history of chronic bladder irritation or history of abdominopelvic radiography (table 1).

- **Occupational exposure to MWFs and other compounds**

Taking the 5-year lag period into account, 35% of cases had been exposed to some type of MWFs (data not shown). Exposure to PAHs other than through MWFs (except for frequency-weighted duration) was higher among cases than among controls. More than half of cases and controls were exposed to chlorinated solvents during their careers. About 15% of cases and controls were exposed to diesel exhaust fumes (table 2). Exposure to non-volatile

nitrosamine was infrequent (4% of cases and 10% of controls) (data not shown). For all the occupational exposures considered, the median duration of exposure was higher among cases than controls, except for the 3 MWFs in the ≥ 25-year time-window (table 2).

The results from conditional logistic regressions, including each occupational exposure index at a time, after adjusting for smoking habits, are presented in Tables 3 and 4. The ORs increased with all the occupational exposure indices used in the 5-25-year time-window, except for synthetic MWFs. For straight MWFs, this increase was statistically significant whatever the exposure indices used – contrasting exposures above and below the median exposure, the OR varied between 5 and 9 (online table 2). For soluble MWFs the increases were statistically significant except for the frequency-weighted duration (*P*=0.076) (table 3) with respect to the median exposures, the OR varied between 2 and 5 (online table 2). With PAHs, ORs increased with the three exposure metrics. Chlorinated solvents also tended to increase the OR, although the increase was not statistically significant for the cumulative index, whatever the exposure indices used. Exposure to diesel exhaust fumes (ever vs. never) was not associated with increased risk (table 4).

Table 5 presents the results of the models combining the effect of exposure to the three types of MWFs adjusted for PAH and for the three smoking metrics. The hypothetical cut-off of 25 years, in line with the empirical induction period, was confirmed by the sensitivity analysis (Online figure 2) [26]. In these joint models, no significant excess risk was observed for soluble or synthetic MWFs. In contrast, the ORs increased with exposure to straight MWFs in the 5-25-year time-window whatever the index considered, and were statistically significant based on the duration of exposure in years and the weighted duration; they were not statistically significant with the cumulative exposure index (table 5). No such increase was observed for older exposures (25+ years).

DISCUSSION

The results presented here suggest that exposure to straight MWFs is associated with an increased risk of bladder cancer between 5 and 25 years after exposure. No increase in bladder cancer was associated with exposure to soluble or synthetic MWFs. Furthermore, no association was found with exposure to MWFs before the cut-off period of 25 years, whatever the type of MWFs considered.

The exposure metrics for different MWF types were defined based on a lag of 5 years and a cut-off period of 25 years prior to the reference date, in line with the empirical induction period for bladder cancer [21-23].

Exposure to MWFs more than 25 years previously was considered as adjustment factors but was not associated with an increased risk of bladder cancer. This is consistent with the hypothesis of an empirical induction period less than 25 years or so implying that any bladder cancer attributable to MWFs would be expected to have occurred in the 25 years following exposure. Those exposures acquired prior to 25 year did not contribute materially to the risk of bladder cancer within the follow-up period; they might have contributed to the risk of bladder cancer before the follow-up period.

When comparing the mortality of the cohort to that of a local reference population, we observed a healthy worker effect which was attenuated with time since hire. We do not believe that this can have a major consequence on the case-control study.

Soluble and synthetic MWFs only gradually came into mainstream use in the last two decades, a fact which might explain the lack of association with these two types of MWFs. However, the upper limits of the confidence intervals of the OR for the soluble MWFs were

close to the OR estimates for straight MWFs in the joint exposure models. Thus, while a risk with soluble oils cannot be excluded, it is certainly less marked than the risk with straight MWFs.

This study follows on from the secondary results of a previous mortality study conducted in French steel-producing factories assessing exposures based on a JEM [5]. A significant excess of mortality from bladder cancer was observed among workers exposed to oil mist and consistent dose-response relationships were found with highest exposure levels in the recorded work histories, durations of exposure and cumulative exposure indices. The results presented here were obtained by a different approach but they confirm the conclusion of our previous study. Moreover, this study estimated the bladder cancer risk in relation to exposure to different types of MWFs, occurring during a more recent period of time and taking smoking habits into account.

Our results are qualitatively supported by previous studies. For example, in 2009, Friesen et al. [6] showed a strong quantitative exposure-response relation between straight MWFs and bladder cancer (RR 2.07, 95% CI 1.19-3.62) in a cohort of autoworkers in Michigan. In 2011, Colt et al. [27] conducted a case-control study drawn from the general population of the New England states, and concluded that exposure to straight MWFs was associated with a significantly increased bladder cancer risk (OR 1.7, 95%CI 1.1-2.5). In both studies, the authors suggested that mineral oils are a bladder carcinogen. Neither study found a statistically significant excess risk of bladder cancer with soluble or synthetic MWFs.

PAHs are generated during pyrolysis or from the incomplete combustion of organic matter. Some areas in steel-producing factories (coking plant, blast furnace, steelmaking plant or foundry) are associated with high PAH exposure [28]. Recent literature reviews and studies have shown an increased risk of bladder cancer in industries where workers are exposed to

PAHs during some processes [8, 28-30]. Here, a semi-quantitative assessment of occupational PAH exposure, MWFs excluded, revealed a significantly increased incidence of bladder cancer among workers exposed to PAHs compared to non-exposed workers (table 4). These conclusions remain valid after adjusting for smoking habits and occupational exposure to MWFs (table 5).

Tobacco consumption contains multiple carcinogens, including PAHs, is the primary risk factor for bladder cancer and is classified by IARC as "carcinogenic to humans" (Group 1) [31]. A strong point is that we collected detailed information on smoking habits and took them into account in the statistical models. As expected from the literature, analysis showed that smoking is significantly associated with a risk of bladder cancer and that this risk increases significantly with the duration of smoking, the average number of cigarettes smoked and the number of pack-years [32-35]. Other non-occupational factors did not have any major effect.

Straight MWFs are mostly composed of mineral oils obtained from crude oil and may contain PAHs, the proportion of which varies depending on the degree of refining [36]. Up to the 1970s, mineral oils were untreated or slightly treated, and contained high concentrations of PAHs. In the mid-80s, in the United-States, the OSHA (Occupational Safety and Health Administration) pressurized cutting oil manufacturers to institute oil refining [10]. Implementation of refining probably decreased the amount of PAH in MWFs. Thus, refining has probably decreased PAH exposure, but our results still show an increased risk of bladder cancer for workers exposed to straight MWFs during the previous 25 years, which corresponds to exposure after the middle of the 1980s. We hypothesize that these results may also be due to the presence of PAHs in straight MWFs. Even if new lubricants are refined since the mid-80, straights MWFs may contain varying amount of PAH, depending on

the PAH enrichment when the MWFs are brought to high temperatures and depending on the degree of regeneration of waste oils.

One of the strengths of the study is that we increased the statistical power of our findings by making use of a pre-existing surrogate exposure estimate that was available for the whole cohort [5] and applying a counter-matching design for control selection [18]. Such *a priori* information is rare and the use of the counter-matching design is relatively original. The counter-matching design has rarely been used in occupational epidemiology [37], except in simulation studies.

In classical nested case-control studies, little information is provided in the model when case and matched controls have similar exposure levels. In contrast, the counter-matching design produces cases and controls with different exposure levels. The bias introduced by this sampling design is accounted for in the conditional multiple logistic regression models by using specific weighting [17]. The advantage of this design is that it improves statistical efficiency. According to Steenland [16], using 3 controls per case with a counter-matching design yields an increase of approximately 25 per cent in relative efficiency compared with random sampling, and it would be roughly equivalent to random sampling using 10 controls. However, in our study, this leads to increased numbers of duplicates in the selected controls. These reused controls belong to smaller risk sets and exposure groups within risk sets. This happens mostly in exposed groups as most cohort members are non-exposed.

Considering the blue and white collar status, only 11 workers (3.3%) were white collars in our case-control study and 96% of the workers not exposed to straight MWF were blue collars. Therefore, this fact cannot entail a major residual confounding.

No cancer registry was available covering the area where the factories participating are located. Cases therefore had to be collected by contacting, inter alia, all 21 hospitals and

clinics in the study area. Two clinics of significant size declined to participate in the study, potentially causing us to miss some eligible cases. However, some of them were retrievable if they were recorded in the national database of requests for exemption of medical expenses related to treatment of long-term diseases. Given that bladder cancer progresses slowly, we are confident that most of the cases were indeed registered in this file.

Over 40% of eligible cases could not be interviewed because there were deceased before interview, they declined to participate or they could not be contacted. No statistical difference in the surrogate exposure was detected between participant and not participant whatever the reason. Thus, the living or the participant status do not seem to bias the exposure assessment.

No atmospheric measurements were available for quantitative assessment of MWF exposure. Instead, the semi-quantitative assessment used was based on detailed exposure questionnaires including individual lifetime work histories and specific questionnaires focusing on all exposing tasks, as well as the firsthand knowledge of the working conditions in the companies of in-house occupational physicians and industrial hygienists, and the safety data sheets available from factories. This retrospective assessment based on the reported job history may be affected by recall bias but there is no reason why this should be different between cases and controls. Anyway, if the misclassification in exposure is non-differential, this would entail an attenuation of the exposure-response relationship; this might explain the lack of association with the ≥ 25 years old.In conclusion, our study highlights the association between the risk of developing bladder cancer and exposure to straight MWFs in the previous 25 years. Our study cannot exclude a relationship with exposure to soluble MWFs, but detects no relationship with synthetic MWFs. These results may be explained by the presence of carcinogens (such as PAH) in new or used mineral oils

and other components of straight oils. Prevention therefore remains necessary in sectors using MWFs.

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CONTRIBUTORSHIP

Each author has contributed to the submitted work as follow: RC and EB drafted the manuscript. RC and EB participated to the data collection. RC managed the data. RC and EB performed statistical analyses. EB reviewed the literature and designed the study. EB, MG, RC, PW and GH participated to the analysis plan. EB developed the job-specific questionnaires. EB managed the exposure assessment. All co-authors collaborated interactively, contributed to the interpretation of the results and discussion, read and approved the final manuscript. Each author believes that the manuscript represents honest work.

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COMPETING INTERESTS

Conflict of interest: none declared.

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Table 1 Subject characteristics in terms of non-occupational factors and unadjusted odds ratio[a] of bladder cancer

		Cases (n=84)			**Controls** (n=251)					
		No.	**%**	**mean (sd)**	**No.**	**%**	**mean (sd)**	**OR[a]**	**95% CI**	***P* value**
Highest education level (years of schooling)										0.449[e]
No qualification		11	13.1		27	10.8		1.53	0.36-6.48	
Less than secondary school (<15 years)		46	54.7		154	61.3		1.48	0.56-3.93	
Secondary school (18 years)		13	15.5		45	17.9		1.25	0.40-3.89	
Higher education (>18 years)		12	14.3		24	9.6		1		
Missing		2	2.4		1	0.4				
Smoking habits[b]										
Smoking status										<0.001[e]
Never smoker		9	10.7		76	30.3		1		
Former smoker		44	52.4		126	50.2		3.84	1.38-10.7	0.010
Current smoker		31	36.9		49	19.5		10.87	3.39-34.9	0.000
Duration of smoking (years)				31.7 (10.4)			26.4 (12.1)			<0.001[f]
Never smoker		9	10.7		76	30.3		1		
1-20 years		10	11.9		44	17.5		2.62	0.72-9.59	0.145
20-40 years		48	57.1		109	43.4		4.39	1.58-12.18	0.005
≥ 40 years		17	20.2		22	8.8		15.29	4.29-54.51	<0.001
Average cigarettes smoked per day				18.1 (9.6)			15.5 (11.4)			0.002[f]
Never smoker		9	10.7		76	30.3		1		
1-10		11	13.1		61	24.3		1.78	0.52-6.16	0.361
10-20		42	50.0		67	26.7		6.66	2.23-19.90	0.001
≥ 20		22	26.2		47	18.7		7.88	2.48-25.03	<0.001
Pack-years				28.4 (18.6)			22.1 (19.7)			<0.001[f]
Never smoker		9	10.7		76	30.3		1		
1-20		24	28.6		94	37.4		2.10	0.69-6.39	0.191
20-40		38	45.2		56	22.3		8.45	2.90-24.64	<0.001
≥ 40		13	15.5		25	10.0		9.37	2.60-33.85	0.001
Time since smoking cessation				12.9 (8.1)			17.9 (11.7)			0.002[f]
Never smoker		9	16.9		76	37.6		1		
≥ 20 years		8	15.1		52	25.8		2.60	0.70-9.72	0.155
10-20 years		18	34.0		33	16.3		4.87	1.10-21.47	0.036
<10 years		18	34.0		41	20.3		5.39	1.33-21.95	0.019
Food consumption at the date of the interview[c]										
Fresh fruit	no	27	32.1		64	25.5		1		
	yes	57	67.9		187	74.5		0.61	0.31-1.23	0.172
Fresh vegetables	no	50	59.5		104	41.4		1		
	yes	34	40.5		147	58.6		0.52	0.26-1.03	0.061
Sweeteners	no	73	86.9		209	83.3		1		
	yes	11	13.1		42	16.7		0.86	0.35-2.10	0.743
Alcohol consumption	no	30	35.7		87	34.7		1		
	yes	54	64.3		164	65.3		0.68	0.34-1.38	0.289
Chronic bladder irritation[d]	no	80	95.2		233	92.8		1		
	yes	4	4.8		18	7.2		0.81	0.19-3.37	0.773
Abdominopelvic radiography	no	57	67.9		167	66.5		1		
	yes	27	32.1		84	33.5		0.62	0.30-1.27	0.190
Familiar history of bladder cancer	no	83	98.8		240	95.6		1		
	yes	1	1.2		11	4.4		0.24	0.02-2.43	0.226

Abbreviations: PAHs, Polycyclic Aromatic Hydrocarbons; MWFs, metalworking fluids; OR, Odds Ratio; 95%CI, 95% confidence intervals

[a] Conditional logistic regression, adapted to counter-matching

[b] Calulations take into account a 5 year-lag

[c] Daily consumption

[d] Urinary schistosomiasis and urolithiasis

[e] From trend analysis

[f] From continous data

Table 2 Exposure to different types of MWF, PAH, Chlorinated solvents and Diesel exhaust fumes, according to 3 continuous exposure indices, for cases and controls

	Cases (n=84)					**Controls** (n=251)				
			Duration (year)	**Frequency-weighted duration** (full-time equivalent year)	**Cumulative index** (intensity.year)			**Duration** (year)	**Frequency-weighted duration** (full-time equivalent year)	**Cumulative index** (intensity.year)
	No.	**%**	**median (*min-max*)[a]**	**median (*min-max*)[a]**	**median (*min-max*)[a]**	**No.**	**%**	**median (*min-max*)[a]**	**median (*min-max*)[a]**	**median (*min-max*)[a]**
Straight MWFs										
5-25-year time-window	13	15.5	14.6 (0.9-20.0)	1 (0.0-13.7)	1.6 (0.0-13.7)	37	14.7	7.3 (0.9-20.0)	0.7 (0.0-13.8)	0.7 (0.0-27.6)
≥ 25-year time-window	21	25.0	4.1 (0.5-23.3)	0.4 (0.0-10.8)	0.4 (0.0-10.8)	82	32.7	5.9 (0.7-23.2)	1.0 (0.0-14.4)	1.1 (0.0-28.8)
Soluble MWFs										
5-25-year time-window	14	16.7	14.6 (0.8-20.0)	1.6 (0.0-13.7)	1.8 (0.1-21.3)	57	22.7	12.4 (0.3-20.2)	1.1 (0.0-15.0)	1.9 (0.0-27.6)
≥ 25-year time-window	14	16.7	5.1 (0.5-14.5)	0.8 (0.0-10.8)	1.2 (0.1-10.8)	83	33.1	7.3 (0.2-25.3)	1.3 (0.0-18.2)	2.3 (0.0-36.4)
Synthetic MWFs										
5-25-year time-window	4	4.8	11.2 (1.1-20.0)	1 (0.8-1.8)	1.3 (1-1.8)	15	6.0	7.4 (0.8-20.0)	1.9 (0.0-15.0)	1.9 (0.0-25.7)
≥ 25-year time-window	5	6.0	4.6 (2.7-9.6)	0.4 (0.0-7.2)	0.4 (0.1-7.2)	23	9.2	9.6 (0.9-25.7)	2.7 (0.2-18.2)	4.1 (0.2-36.4)
PAHs[b,c]	34	40.5	14.3 (1.1-33.4)	0.7 (0.0-22.9)	1.6 (0.0-45.9)	80	31.9	7.5 (0.3-39.6)	0.9 (0.0-24.6)	1.2 (0.0-73.9)
Chlorinated solvent[b]	43	51.2	21.4 (1.1-37.3)	1.0 (0.1-28.0)	1.1 (0.1-56.0)	136	54.2	16.5 (0.2-39.6)	0.9 (0.0-8.7)	1.0 (0.0-17.5)
Diesel exhaust fumes[b,d]	13	15.5	13.0 (3.3-36.1)	-	-	38	15.1	10.1 (0.6-39.2)	-	-

Abbreviations: PAHs, Polycyclic Aromatic Hydrocarbons; MWFs, metalworking fluids

[a] Workers who were never exposed were excluded when calculating the median, the minimum and the maximum

[b] Calculated with a 5-year lag

[c] PAHs except MWFs

[d] Frequency and intensity not determined

Table 3 Estimated odds ratio[a] for the association between bladder cancer and MWF exposure for 3 continuous exposure indices, adjusted for duration of smoking, average cigarettes smoked per day and time since smoking cessation

	Duration (year)			**Frequency-weighted duration** (full-time equivalent year)			**Cumulative index** (intensity.year)		
	OR[a]	**95% CI**	***P* value[b]**	**OR[a]**	**95% CI**	***P* value[b]**	**OR[a]**	**95% CI**	***P* value[b]**
Straight MWFs									
5-25-year time-window	1.12	1.03-1.22	0.005	1.36	1.02-1.82	0.019	1.24	1.00-1.53	0.022
≥ 25-year time-window	0.97	0.89-1.06	0.721	0.90	0.66-1.22	0.756	0.89	0.71-1.11	0.859
Soluble MWFs									
5-25-year time-window	1.08	1.01-1.16	0.011	1.12	0.96-1.30	0.076	1.14	1.01-1.28	0.014
≥ 25-year time-window	0.91	0.82-1.01	0.961	0.91	0.73-1.13	0.808	0.90	0.78-1.05	0.915
Synthetic MWFs									
5-25-year time-window	1.02	0.86-1.21	0.400	0.81	0.47-1.38	0.785	0.90	0.60-1.36	0.689
≥ 25-year time-window	0.95	0.80-1.13	0.726	1.13	0.86-1.49	0.181	1.03	0.84-1.26	0.388

Abbreviations: MWFs, metalworking fluids; OR, Odds Ratio; 95%CI, 95% confidence intervals

[a] Conditional logistic regression adapted to counter-matching, for each type of MWFs

[b] One-tailed p-value

Table 4 Estimated odds ratio[a] for the association between bladder cancer and occupational exposure for 3 continuous exposure indices, adjusted for duration of smoking, average cigarettes smoked per day and time since smoking cessation, with a 5 year-lag.

	Duration (year)			**Frequency-weighted duration** (full-time equivalent year)			**Cumulative index** (intensity.year)		
	OR[a]	**95% CI**	***P* value[b]**	**OR[a]**	**95% CI**	***P* value[b]**	**OR[a]**	**95% CI**	***P* value[b]**
PAHs[c]	1.05	1.01-1.09	0.004	1.12	1.03-1.22	0.005	1.04	1.00-1.08	0.025
Chlorinated solvent	1.02	1.00-1.05	0.046	1.13	0.99-1.28	0.037	1.07	0.99-1.16	0.054
Diesel exhaust fumes[d]	1.02	0.97-1.07	0.190	-	-	-	-	-	-

Abbreviations: PAHs, Polycyclic Aromatic Hydrocarbons; MWFs, metalworking fluids; OR, Odds Ratio; 95%CI, 95% confidence intervals

[a] Each OR is the result of conditional logistic regression, adapted to counter-matching

[b] One-tailed p-value

[c] PAHs except in MWFs

[d] Frequency and intensity not determined

Table 5 Estimated odds ratio for the association between bladder cancer and occupational exposure to MWFs, PAHs, and smoking from three multiple conditional logistic regressions adapted to the counter-matching design[a]

	Duration (year)			**Frequency-weighted duration** (full-time equivalent year)			**Cumulative index** (intensity.year)		
	OR	**95% CI**	***P* value[b]**	**OR**	**95% CI**	***P* value[b]**	**OR**	**95% CI**	***P* value[b]**
Straight MWFs									
5-25-year time-window	1.13	1.02-1.25	0.012	1.44	0.97-2.14	0.034	1.18	0.92-1.51	0.096
≥ 25-year time-window	0.99	0.89-1.10	0.558	0.94	0.66-1.34	0.638	0.93	0.72-1.19	0.724
Soluble MWFs									
5-25-year time-window	1.06	0.98-1.15	0.059	1.07	0.86-1.33	0.285	1.12	0.97-1.30	0.055
≥ 25-year time-window	0.87	0.76-0.99	0.980	0.82	0.61-1.10	0.912	0.90	0.74-1.10	0.853
Synthetic MWFs									
5-25-year time-window	0.96	0.76-1.23	0.614	0.82	0.40-1.72	0.698	0.82	0.52-1.28	0.811
≥ 25-year time-window	0.96	0.78-1.18	0.656	1.31	0.88-1.95	0.094	1.15	0.87-1.50	0.163
PAHs[c]	1.05	1.01-1.09	0.005	1.12	1.03-1.22	0.004	1.04	1.00-1.08	0.019
Duration of smoking	1.04	1.01-1.08	0.003	1.04	1.01-1.08	0.002	1.05	1.02-1.08	0.001
Average cigarettes smoked per day	1.02	0.99-1.05	0.131	1.02	0.99-1.06	0.091	1.02	0.99-1.06	0.089
Time since smoking cessation	0.97	0.94-1.01	0.088	0.98	0.94-1.01	0.102	0.98	0.94-1.01	0.114

Abbreviations: PAHs, Polycyclic Aromatic Hydrocarbons; MWFs, metalworking fluids; OR, Odds Ratio; 95%CI, 95% confidence intervals

[a] Calculations consider a 5 year-lag

[b] One-tailed p-value

[c] PAHs except in MWFs, same method of calculation as the MWF metric considered in the model