Diesel exhaust and bladder cancer risk by pathologic stage and grade subtypes

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ABSTRACT

Background: The International Agency for Research on Cancer (IARC) classifies diesel engine exhaust as carcinogenic to humans based on sufficient evidence for lung cancer. IARC noted, however, an increased risk of bladder cancer (based on limited evidence).
Objective: To evaluate the association between quantitative, lifetime occupational diesel exhaust exposure and

risk of urothelial cell carcinoma of the bladder (UBC) overall and according to pathological subtypes.

Methods: Data from personal interviews with 1944 UBC cases, as well as formalin-fixed paraffin-embedded tumor tissue blocks, and 2135 controls were pooled from two case-control studies conducted in the U.S. and Spain. Lifetime occupational histories combined with exposure-oriented questions were used to estimate cumulative exposure to respirable elemental carbon (REC), a primary surrogate for diesel exhaust. Unconditional logistic regression and two-stage polytomous logistic regression were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs), adjusting for smoking and other risk factors.

Results: Exposure to cumulative REC was associated with an increased risk of UBC; workers with cumulative REC > 396 μg/m^3-years had an OR of 1.61 (95% CI, 1.08–2.40). At this level of cumulative exposure, similar results were observed in the U.S. and Spain, OR = 1.75 (95% CI, 0.97–3.15) and OR = 1.54 (95% CI, 0.89–2.68), respectively. In lagged analysis, we also observed a consistent increased risk among workers with cumulative REC > 396 μg/m^3-years (range of ORs = 1.52–1.93) for all lag intervals evaluated (5–40 years). When we accounted for tumor subtypes defined by stage and grade, a significant association between diesel exhaust exposure and UBC was apparent (global test for association p = 0.0019).

Conclusions: Combining data from two large epidemiologic studies, our results provide further evidence that diesel exhaust exposure increases the risk of UBC.

1. Introduction

In 2012, the International Agency for Research on Cancer (IARC) classified diesel engine exhaust as carcinogenic to humans (Group 1) based on sufficient positive evidence for lung cancer. The working group also noted a positive association between diesel exhaust and risk of bladder cancer based on limited evidence in humans (Benbrahim-Tallaa et al., 2012; IARC, 2014). The link between diesel exhaust and bladder cancer risk has largely come from studies of specific occupations where diesel exhaust exposure was inferred (including truck drivers, mechanics, railroad workers, and operators of heavy equipment). Boffetta & Silverman conducted a meta-analysis of diesel exhaust exposure among occupations with high exposure and found that the summary relative risks for bladder cancer were 1.23 (95% CI, 1.12–1.36) for any exposure and 1.44 (95% CI, 1.18–1.76) for high exposure to diesel exhaust (Boffetta and Silverman, 2001). Few studies have been able to go beyond job title to assess lifetime individual diesel exhaust exposure (IARC, 2014), and none have incorporated quantitative estimates for respirable elemental carbon (REC), the primary surrogate for diesel exhaust exposure (Birch and Cary, 1996). Further, little is known about the mechanisms by which diesel exhaust might lead to bladder cancer. Although new molecular subtypes of bladder cancer are being uncovered, alterations in TP53 and FGFR3 still delineate the two major subtypes, where *FGFR3* alterations are commonly found in non-muscle invasive bladder cancer and *TP53* alterations are common in the more aggressive subtype, muscle-invasive bladder cancer (stage T2 +) (Knowles and Hurst, 2015).

Here we examined the association between quantitative, lifetime occupational diesel exhaust exposure and risk of urothelial cell carcinoma (UBC) in two large case-control studies in the United States (U.S.) and Spain. In addition, we evaluated heterogeneity in the diesel exhaust-UBC relationship by tumor subtypes including stage, grade, as well as expression and mutation of *TP53* and *FGFR3*.

2. Methods

2.1. Study populations

Data from two UBC case-control studies were included in the current analysis. The New England Bladder Cancer Study (NEBCS), a large population-based case-control study, included 1213 cases and 1418 controls from three New England states. Cases in the NEBCS were all patients with histologically confirmed UBC newly diagnosed between 2001 and 2004 among residents of Maine, New Hampshire, and Vermont, ages 30 to 79 years. A total of 1213 UBC patients were ascertained through hospital pathology departments and hospital and state cancer registries and interviewed (65% of eligible cases were interviewed). Control subjects were selected randomly from state Department of Motor Vehicle records (age 30–64 years) and Centers for Medicare and Medicaid Services beneficiary records (age 65–79 years), frequency matched to case patients on state, sex, and five-year age group at diagnosis/interview. A total of 1418 (594 Department of Motor Vehicle, 824 Centers for Medicare and Medicaid Services) control subjects (65% of eligible Department of Motor Vehicle and 65% of eligible Centers for Medicare and Medicaid Services control subjects) were interviewed.

The Spanish Bladder Cancer Study (SBCS), a large hospital-based case-control study of 1219 cases and 1271 controls, was conducted in 18 hospitals from 5 areas in Spain. Cases in the SBCS were patients newly diagnosed with histologically confirmed UBC in 1998–2001, aged 21–80 years. A total of 1219 cases were interviewed (84% of eligible cases). For each case, one control was selected and individually matched on age (within 5 years) at diagnosis/interview, gender, race/ethnicity and hospital. Controls were selected from patients admitted to the same hospital around the same time as the cases for diseases/conditions unrelated to the exposures under study. A total of 1271 controls were interviewed (88% of eligible controls).

A standardized histopathology review to assign stage and grade was carried out for each study by a study pathologist (A.S. for NEBCS and J.L. and Y.A. for SBCS). Tumors were staged according to TNM criteria of the American Joint Commission on Cancer (Sobin, 2009; Sobin, 2002) and for grade according to both the 1973 WHO and 2004 WHO/ISUP criteria (Eble et al., 2004; Mostfi, 1973). For both studies, participants were interviewed by trained interviewers using the same instrument, a computer-assisted personal interview. We obtained detailed information on demographics, use of tobacco products, lifetime occupational histories, family history of cancer, dietary factors, and residential histories.

The current analysis includes 1944 newly diagnosed UBC cases and 2135 controls among eligible males with occupational histories. Women were excluded due to few diesel-exposed jobs. Representative high diesel-exposed jobs among men were heavy and tractor-trailer truck drivers, bus, truck and heavy equipment engine mechanics and repairers, and material moving equipment operators (e.g., grader, dozer, and scraper and industrial truck and tractor). Analytic exclusions for each study were as follows: in the NEBCS, of the 1213 interviewed cases, we excluded 20 patients who were found not to have UBC based on a pathologist's review, 23 patients with non-urothelial carcinomas and 273 female patients, leaving 897 cases for analysis. Among the 1418 interviewed controls, we excluded 9 participants based on incomplete work histories, and 372 females, leaving 1037 controls for analysis. In the SBCS, among 1219 interviewed cases, we excluded 16 patients with non-urothelial carcinomas, 36 with incomplete work histories and 120 female patients, leaving 1047 cases for analysis. Among the 1271 interviewed controls, we excluded 50 participants based on incomplete work histories and 123 females, leaving 1098

controls for analysis.

All participants provided written consent. The study protocol was approved by all appropriate institutional review boards.

2.1.1. Tumor markers

Formalin-fixed paraffin-embedded tumor tissue blocks assembled as tissue microarrays (TMAs) from UBC cases enrolled in the Maine and Vermont components of the NEBCS and individual slides in the SBCS were used to examine protein expression of TP53 and FGFR3. Details of the immunohistochemical (IHC) methods for each marker have been published elsewhere (for NEBCS: p53 (Lenz et al., 2012) and FGFR3 (Figueroa et al., 2015); for SBCS: p53 (Balbas-Martinez et al., 2013) and FGFR3 (Amaral et al., 2012)). Antibodies used for P53 and FGFR3 expression were the same in both studies. Immunohistochemical scoring for P53 and FGFR3 expression in both the NEBCS and SBCS have been previously described (Lenz et al., 2012; Figueroa et al., 2015; Balbas-Martinez et al., 2013; Amaral et al., 2012). Briefly, for TP53, quantitative scoring models were used to compute the percentage of positive cells. In addition, immunoreactivity was scored according to levels of intensity and a histoscore was calculated as the product of intensity and percentage of positive cells. FGFR3 expression was found to be both cytoplasmic and membranous. Cells were scored according to staining intensity and location (NEBCS) as well as the proportion of reactive cells (SBCS).

Serial sections from tumor blocks (5-μm in NEBCS and 10-μm in SBCS) were cut to extract nucleic acids for characterization of TP53 and FGFR3 somatic mutations. In the NEBCS, tumor regions as annotated on digital images were hand macrodissected from three 5 μm sections and placed into 1.5 ml microcentrifuge tubes. DNA was isolated using the phenol-based AutoGenprep 245 T Animal Tissue DNA Extraction Kit (Autogen) according to the manufacturer's method. Yield and purity were determined by NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). In the SBCS, areas containing > 50% tumor cells were manually macrodissected from three to five consecutive 10-μm sections and DNA was extracted using the DNeasy tissue kit (Qiagen GmbH, Hilden, Germany). Exons 7, 10, and 15 of FGFR3 and Exons 5 to 8 of TP53, which harbor the majority of mutations observed in bladder tumors, were amplified and sequenced using the SnapSHOT assay (for 11 known FGFR3 mutations) or double stranded Sanger Sequencing using Big Dye v.3.1 (Thermo Fisher Scientific) in both studies (described elsewhere) (Balbas-Martinez et al., 2013; Hernandez et al., 2005; Hafner et al., 2006).

In the current analysis, immunophenotype was dichotomized as follows: "P53 IHC" (≤ 5% of positive cells and > 5% positive cells), "P53 Histoscore" (greater and less than the median histoscore), "FGFR3 IHC" (high expression/strong staining vs. intermediate/weak). Mutation status ("TP53 Mut" and "FGFR3 Mut") was dichotomized as the presence or absence of any observed mutation.

Tumor characteristics were available on a subset of all analytic cases (58.8% of cases for P53 immunohistochemistry, 21.7% of cases for TP53 mutation, 44.4% of cases for FGFR3 mutation, and 46.4% of cases for FGFR3 immunohistochemistry).

2.2. Lifetime occupational histories and diesel exhaust exposure assessment

The main metric of analysis in the current study was cumulative respirable elemental carbon (REC) exposure. The cumulative exposure calculation was limited to jobs with a probability of exposure ≥ 50% (jobs where the estimated proportion of workers exposed to diesel exhaust was ≥ 50%). This metric was calculated by summing the product of intensity of exposure, frequency of exposure and duration of exposure over all jobs. Exposure intensity was assessed on a continuous scale as the estimated average REC exposure level (μg/m^3) for a given job based on an extensive review of the diesel exhaust occupational health literature that identified diesel-exposed tasks, jobs, industries, and secular changes in the prevalence of diesel engine use (Pronk et al.,

2009). Frequency (hours/week) and duration (years) of exposure were collected in both studies using lifetime occupational histories (all jobs a subject held for at least 6 months since age 16) (Colt et al., 2011; Samanic et al., 2008). In the occupational histories, participants were queried about location (indoor/outdoor), job dates, activities performed, products/services made/provided, and equipment/chemicals handled. For each job, two additional questions were asked about working near engines or about smelling engine exhaust, including diesel. Additional exposure-oriented questions were asked to obtain detailed information on diesel exposure for subjects responding "yes" to the engine questions and for those in jobs or industries with potential for diesel exhaust.

In the NEBCS, an industrial hygienist blindly assigned probability and intensity of diesel exposure for each job (while frequency and duration came from direct participant response). Based on the NEBCS, decision rules were extracted from the industrial hygienists estimates and questionnaire response patterns using a classification tree model (CART) in order to obtain estimates of probability (< 5%, 5–49%, 50–79%, ≥ 80%), intensity (< 0.25, 0.25 to < 5, 5 to < 20, and ≥ 20 μg/m^3 REC), and frequency (< 0.25, 0.25– < 8, 8– < 20, ≥ 20 h/ week) of diesel exhaust exposure. The decision rules from the NEBCS were then applied to questionnaire response patterns in the SBCS in order to obtain estimates of probability, intensity, and frequency of exposure to diesel exhaust (Friesen et al., 2016). An additional focused expert review of a subset of jobs was employed in the SBCS when we were less confident in the classification tree models' estimates or the classification tree estimates of different metrics were discordant.

A list of the main job titles contributing to diesel exposure in the two study populations is provided in Appendix A.1.

2.3. Statistical analysis

Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the relationship between diesel exhaust and UBC. Models were adjusted for age, race, study, smoking, and non-diesel exposed high-risk occupations for UBC (Silverman et al., 2018). Exposure categories were defined in quartiles with the top quartile further divided at the > 75-90th percentile, > 90–95th percentile, > 95th percentile based on the distribution of exposure in the controls. We also evaluated the risk of bladder cancer for cumulative REC exposure by decade of first diesel exposed job, as well as by various lag periods (5–40 years). We calculated tests for linear trend using the Wald test, treating the median value for each category (including unexposed) among control subjects as continuous. P-values for interaction were computed by comparing nested models with and without the cross-product terms based on a likelihood ratio test.

We used a novel two-stage polytomous logistic regression model (Zhang et al., 2018) as modified from Chatterjee (Chatterjee, 2004) to evaluate the relationship between diesel exhaust and UBC risk while assessing heterogeneity by tumor subtypes as defined by stage (Ta, T1, T2 +), grade (G1, G2, G3), p53 and FGFR3 immunohistochemical (IHC) phenotype (positive vs. negative expression, see supplementary methods for dichotomization) and TP53 and FGFR3 somatic mutations (dichotomized as the presence/absence of mutation). This method allows for efficient testing of exposure-disease associations in the presence of tumor subtype heterogeneity defined by multiple disease characteristics, while accounting for multiple comparisons and missing data on tumor markers/features. The model provides a global test for association (diesel exhaust-UBC) in the presence of heterogeneity by tumor features, as well as individual tests for heterogeneity of each tumor marker, adjusting for the other markers in the model. In the current analysis, we fitted separate models for each tumor marker (TP53 and FGFR3) with stage and grade. The final model included only the significant tumor markers, stage and grade.

3. Results

Table 1 shows the characteristics of male cases of UBC and controls in both studies. Cases who were exposed to diesel exhaust had a median of 26 years of exposure while controls had a median of 27 years of exposure; cumulative REC exposure among controls was more than twice as high in the SBCS compared to the NEBCS. Compared with cases from the NEBCS, incident tumors of cases in the SBCS were more likely to be muscle-invasive (23.0% vs. 13.2%) and have a higher grade.

No significant associations between ever exposure, duration (alone), or intensity of exposure (alone) and risk of bladder cancer were observed (data not shown). Table 2 shows the ORs and 95% CIs for cumulative REC exposure, by study and stratified by smoking status. Cumulative REC was associated with an increased risk of UBC, with the highest level of cumulative REC > 396 µg/m^3-years having an OR of 1.61 (95% CI, 1.08–2.40) compared to unexposed subjects (p-trend = 0.08). At this level of cumulative exposure, nonsignificant, elevated risks were observed in both the NEBCS (OR = 1.75; 95% CI, 0.97–3.15) and SBCS (OR = 1.54; 95% CI, 0.89–2.68). Among never smokers, we observed a trend in risk with increasing cumulative REC (p-trend = 0.05); subjects with levels of cumulative REC > 254 µg/m^3-years had an OR of 1.94 (95% CI, 0.91–4.12), while little or no excess risks were seen among former and current smokers (Table 2). The p-value for interaction between diesel exhaust and smoking among all participants was not statistically significant (p = 0.49), although evidence of an interaction was more pronounced in New England than in Spain (p-value for interaction in NEBCS = 0.14 and SBCS = 0.79).

Time-period analyses by decade of first diesel exhaust-exposed job showed a statistically significant increased risk among heavily exposed men who were first exposed in the 1950s in both studies (overall cumulative REC > 396 µg/m^3-years: OR = 1.97, 95% CI, 1.05–3.72)

(Table 3). To accommodate this secular effect, we estimated the exposure-response by various lag intervals (Table 4). We observed a consistent positive association between cumulative REC > 396 µg/m^3-years (vs. unexposed) and bladder cancer risk for all lag intervals evaluated: lagged 5-years, OR = 1.52, 95% CI, 1.01–2.29; lagged 10-years, OR = 1.65, 95% CI, 1.07–2.55; lagged 15-years, OR = 1.73, 95% CI, 1.10–2.71; lagged 20-years, OR = 1.93, 95% CI, 1.19–3.15; lagged 25-years, OR = 1.83, 95% CI, 1.06–3.18; lagged 30-years, OR = 1.79, 95% CI, 0.93–3.45; lagged 35-years, OR = 1.92, 95% CI, 0.85–4.31; lagged 40-years, OR = 1.66, 95% CI, 0.68–4.05. In contrast, a gradient in risk with increasing cumulative REC was only apparent when exposure was lagged 40 years.

After controlling for stage and grade using the two-stage regression model, we observed no heterogeneity in the relationship between diesel exhaust exposure and UBC when considering tumor subtypes characterized by expression or mutation of *TP53* and *FGFR3* (see Appendix A.2 for all *TP53* and *FGFR3* case-case parameters p > 0.05). When we account for heterogeneity in tumor subtypes as defined by stage and grade alone, we observed a significant association between diesel exhaust exposure and UBC (global test for association for diesel exhaust exposure and UBC in the presence of heterogeneity by stage and grade: p = 0.0019; global test for heterogeneity: p = 0.0196). When we explored the increased risk associated with cumulative REC by stage (Ta, T1, T2 +) and grade (G1, G2, G3/G4), we observed a consistent association between cumulative REC across stage and grade categories (Fig. 1). The increased risk associated with cumulative REC by pathologic stage was stronger among those with muscle-invasive bladder cancer: T2 + OR = 2.03 (95%CI, 1.11–3.74), p = trend = 0.014 as well as for those with low grade tumors (G1, OR = 2.08 (95%CI, 1.28, 3.38), p-trend = 0.002 (Appendix A.3 and Fig. 1).

Table 1
Descriptive characteristics of male UBC cases and controls in the New England and Spanish Bladder Cancer Studies.

Characteristic	Total		New England		Spain	
	Cases n (%) N = 1944	Controls n (%)N = 2135	Cases n (%)N = 897	Controls n (%) N = 1037	Cases n (%) N = 1047	Controls n (%) N = 1098
Age						
< 65	762 (39.2)	894 (41.9)	377 (42.0)	421 (40.6)	385 (36.8)	473 (43.1)
65–74	796 (40.9)	870 (40.7)	340 (37.9)	409 (39.4)	456 (43.5)	461 (41.9)
75 +	386 (19.9)	371 (17.4)	180 (20.0)	207 (20.0)	206 (19.7)	164 (14.9)
Smoking Status						
Never	170 (8.7)	534 (25.0)	114 (12.7)	307 (29.6)	56 (5.3)	227 (20.7)
Occasional	54 (2.7)	107 (5.0)	19 (2.1)	24 (2.3)	35 (3.3)	83 (7.6)
Regular						
Former	968 (49.8)	1058 (49.6)	484 (54.0)	556 (53.6)	484 (46.2)	502 (45.7)
Current	745 (38.3)	429 (20.1)	279 (31.1)	149 (14.4)	466 (44.5)	280 (25.5)
Missing	7 (< 1)	7 (< 1)	1 (< 1)	1 (< 1)	6 (< 1)	6 (< 1)
Stage						
Carcinoma in situ	50 (2.6)	–	50 (5.6)	–	0	–
Ta	1215 (62.5)	–	568 (63.3)	–	647 (61.8)	–
T1	281 (14.5)	–	159 (17.7)	–	122 (11.7)	–
T2 +	359 (18.5)	–	118 (13.2)	–	241 (23.0)	–
Grade						
G1	712 (37.6)	–	397 (44.3)	–	315 (30.1)	–
G2	473 (25.0)	–	176 (20.8)	–	297 (28.4)	–
G3	664 (35.1)	–	266 (31.4)	–	398 (38.0)	–
Probability of diesel exposure						
Unexposed	760 (39)	857 (40)	240 (27)	319 (31)	520 (50)	538 (50)
< 50% exposure probability	258 (13)	245 (12)	154 (17)	153 (15)	104 (10)	92 (8)
≥50% exposure probability	926 (48)	1033 (48)	503 (56)	565 (55)	423 (40)	468 (43)
Duration of diesel-exposed jobs (median years, IQR)	26 (9–45)	27 (9–46)	23 (8–48)	24 (8–47)	28 (11–43)	29 (11–45)
Cumulative Diesel Exposure (µg/m^3 REC-years) (median, IQR)	26 (6–96)	26 (5–98)	16 (3–85)	15 (3–69)	38 (10–112)	39 (10–116)

Percentages may not sum to 100 due to rounding.

Table 2

Odds ratios (ORs) and 95% confidence intervals (CIs) for cumulative REC exposure overall, by study and smoking status.

Exposure Metric	Ca	Co	Overall OR* 95% CI	Ca	Co	NEBCS OR* 95% CI	Ca	Co	SBCS OR* 95% CI
Cumulative REC (µg/m³-years)¶									
Overall									
Unexposed	760	857	1.0	240	319	1.0	520	538	1.0
> 0–5	225	258	1.01 (0.81, 1.27)	161	186	1.08 (0.81, 1.45)	64	72	0.86 (0.59, 1.26)
> 5–25	232	258	0.96 (0.78, 1.20)	128	140	0.95 (0.69, 1.3)	104	118	1.00 (0.73, 1.36)
> 25–98	241	258	1.09 (0.88, 1.35)	101	124	0.90 (0.64, 1.25)	140	134	1.28 (0.96, 1.71)
> 98–254	126	154	0.95 (0.72, 1.24)	62	69	1.00 (0.67, 1.50)	64	85	0.91 (0.63, 1.33)
> 254–396	36	53	0.81 (0.51, 1.29)	19	22	1.04 (0.53, 2.05)	17	31	0.69 (0.36, 1.30)
> 396	66	52	1.61 (1.08, 2.40)	32	24	1.75 (0.97, 3.15)	34	28	1.54 (0.89, 2.68)
p-trend			0.08			0.09			0.33
Among Never Smokers									
Unexposed	71	226	1.0	42	117	1.0	29	109	1.0
> 0–25	42	142	0.81 (0.51, 1.29)	34	104	0.83 (0.48, 1.44)	8	38	0.84 (0.33, 2.14)
> 25–254	25	93	1.03 (0.59, 1.78)	13	41	0.96 (0.45, 2.07)	12	52	1.11 (0.49, 2.49)
> 254	14	27	1.94 (0.91, 4.12)	8	11	2.47 (0.85, 7.17)	6	16	1.66 (0.55, 5.04)
p-trend			0.05			0.07			0.31
Among Former Smokers									
Unexposed	364	410	1.0	126	153	1.0	238	257	1.0
> 0–25	216	258	0.96 (0.75, 1.23)	151	179	0.95 (0.68, 1.33)	65	79	0.95 (0.64, 1.40)
> 25–254	179	186	1.22 (0.94, 1.58)	85	106	1.02 (0.70, 1.50)	94	80	1.44 (1.00, 2.08)
> 254	50	50	1.25 (0.81, 1.91)	26	23	1.53 (0.82, 2.86)	24	27	1.05 (0.58, 1.91)
p-trend			0.19			0.14			0.61
Among Current Smokers									
Unexposed	295	171	1.0	65	44	1.0	230	127	1.0
> 0–25	185	97	1.12 (0.81, 1.55)	99	32	1.79 (1.01, 3.17)	86	65	0.81 (0.54, 1.22)
> 25–254	155	102	0.91 (0.66, 1.26)	62	42	0.87 (0.49, 1.53)	93	60	0.93 (0.62, 1.40)
> 254	37	23	1.00 (0.57, 1.78)	17	11	1.04 (0.43, 2.50)	20	12	1.03 (0.48, 2.21)
p-trend			0.77			0.46			0.82
p$_{smoking}$-interaction	0.49			0.14			0.79		

Abbreviations: Odds ratio (OR), confidence interval (CI), Cases (Ca), Controls (Co), respirable elemental carbon (REC).

*Adjusted for age, race, study, smoking status, and non-diesel exposed high-risk occupations.

¶Cumulative REC categories defined by 25th (5 µg/m3-years), 50th (25 µg/m3-years), 75 (98 µg/m3-years) with the top quartile further divided at the 90th (254 µg/m3-years) and 95th percentile (396 µg/m3-years) among exposed controls.

Table 3

Odds ratios and 95% confidence intervals for cumulative REC exposure by decade of first diesel exposed job.

Cumulative REC (µg/m³-years)*	Decade of 1st diesel exposed job¶											
	< 1950			1950s			1960s			1970s		
	Ca	Co	OR** 95% CI	Ca	Co	OR** 95% CI	Ca	Co	OR** 95% CI	Ca	Co	OR** 95% CI
Unexposed	760	857	1.0	760	857	1.0	760	857	1.0	760	857	1.0
> 0–25	35	34	1.11 (0.66, 1.86)	150	155	1.08 (0.83, 1.42)	115	132	0.88 (0.66, 1.18)	83	109	0.84 (0.60, 1.17)
> 25–254	52	41	1.39 (0.89, 2.18)	120	144	0.96 (0.72, 1.27)	116	137	0.97 (0.73, 1.30)	60	75	0.96 (0.66, 1.41)
> 254–396	8	15	0.63 (0.25, 1.55)	12	16	0.93 (0.42, 2.03)	6	11	0.67 (0.23, 1.97)	8	9	0.84 (0.30, 2.39)
> 396	17	14	1.50 (0.71, 3.18)	25	20	1.97 (1.05, 3.72)	19	14	1.40 (0.67, 2.90)	5	4	1.21 (0.29, 5.06)
p-trend			0.44			0.08			0.51			0.96

*Exposure categories as unexposed, > 0–50th percentile, > 50–90th percentile, > 90–95th percentile, and > 95th percentile.

**Adjusted for age, race, study, smoking status, and non-diesel exposed high-risk occupations.

¶Estimates for 1980s and 1990s not shown due to few exposed cases.

4. Discussion

Findings from these two large, case-control studies show a significant positive association between heavy exposure to diesel exhaust and UBC risk, providing further evidence that diesel exhaust causes bladder cancer. These studies were designed, in part, to improve upon prior limitations in exposure assessment for diesel exhaust and fill in critical gaps in the literature linking this exposure to UBC by incorporating quantitative levels of REC based on expert assessment and lifetime occupational histories.

The link between diesel exhaust exposure and UBC risk was first suggested in diesel exhaust-exposed truck drivers in Detroit in 1983 (Silverman et al., 1983). Since then, several case-control studies

reported increased risk associated with employment in diesel-exposed occupations such as truck drivers, bus drivers, railroad workers and mechanics (IARC, 2014; Silverman et al., 1986). More convincing evidence comes from studies that have estimated risk for workers with known exposure to diesel exhaust, rather than inferring subjects were exposed because they held possible diesel-exposed jobs. In these studies, exposure to diesel exhaust is consistently related to increased risk of UBC in the range of 1.2–1.7 (Latifovic et al., 2015; Pesch et al., 2000; Richardson et al., 2007; Risch et al., 1988; Siemiatycki et al., 1994). This finding is in line with our finding of a 61% increase in risk for UBC at the high end of exposure. Results were consistent in the two studies, with slightly higher cumulative REC observed in Spain compared to New England due to differences in reported work patterns (median

Table 4

Odds ratios and 95% confidence intervals for cumulative REC exposure lagged by various intervals.

Cumulative REC (µg/m³-years)*	Ca	Co	OR** 95% CI
Lag-5 years			
Unexposed	773	869	1.0
> 0–5	222	256	0.99 (0.79, 1.24)
> 5–25	233	263	0.95 (0.77, 1.18)
> 25–98	239	261	1.06 (0.85, 1.31)
> 98–254	123	138	1.03 (0.78, 1.36)
> 254–396	35	53	0.78 (0.49, 1.24)
> 396	61	50	1.52 (1.01, 2.29)
p-trend			0.12
Lag-10 years			
Unexposed	785	884	
> 0–5	225	256	1.01 (0.81, 1.27)
> 5–25	237	270	0.93 (0.75, 1.16)
> 25–98	233	264	1.03 (0.83, 1.27)
> 98–254	115	121	1.11 (0.83, 1.48)
> 254–396	36	52	0.81 (0.51, 1.29)
> 396	55	43	1.65 (1.07, 2.55)
p-trend			0.053
Lag-15 years			
Unexposed	805	901	1.0
> 0–5	231	269	0.97 (0.78, 1.21)
> 5–25	242	259	0.98 (0.79, 1.21)
> 25–98	221	266	0.96 (0.77, 1.19)
> 98–254	107	109	1.12 (0.83, 1.51)
> 254–396	29	47	0.73 (0.44, 1.2=0)
> 396	51	39	1.73 (1.10, 2.71)
p-trend			0.057
Lag-20 years			
Unexposed	835	941	1.0
> 0–5	231	281	0.92 (0.74, 1.15)
> 5–25	234	250	0.98 (0.79, 1.22)
> 25–98	227	244	1.10 (0.88, 1.36)
> 98–254	94	103	1.03 (0.75, 1.41)
> 254–396	19	40	0.58 (0.33, 1.05)
> 396	46	31	1.93 (1.19, 3.15)
p-trend			0.050
Lag-25 years			
Unexposed	898	1003	1.0
> 0–5	243	281	0.95 (0.77, 1.17)
> 5–25	219	235	1.03 (0.83, 1.28)
> 25–98	188	233	0.91 (0.73, 1.14)
> 98–254	82	86	1.14 (0.81, 1.59)
> 254–396	21	27	0.99 (0.54, 1.82)
> 396	35	25	1.83 (1.06, 3.18)
p-trend			0.039
Lag-30 years			
Unexposed	965	1093	1.0
> 0–5	244	278	0.95 (0.77, 1.17)
> 5–25	210	220	1.09 (0.88, 1.37)
> 25–98	164	191	0.99 (0.78, 1.26)
> 98–254	59	70	1.02 (0.70, 1.49)
> 254–396	20	20	1.18 (0.61, 2.29)
> 396	24	18	1.79 (0.93, 3.45)
p-trend			0.09
Lag-35 years			
Unexposed	1082	1225	1.0
> 0–5	235	247	1.05 (0.85, 1.30)
> 5–25	158	205	0.92 (0.72, 1.16)
> 25–98	135	134	1.13 (0.86, 1.47)
> 98–254	46	50	1.14 (0.74, 1.76)
> 254–396	12	19	0.95 (0.44, 2.06)
> 396	18	10	1.92 (0.85, 4.31)
p-trend			0.12
Lag-40 years			
Unexposed	1207	1386	1.0
> 0–5	198	207	1.06 (0.84, 1.33)
> 5–25	149	154	1.15 (0.89, 1.48)
> 25–98	79	91	1.00 (0.72, 1.40)
> 98–254	32	38	1.23 (0.74, 2.03)
> 254–396	8	5	1.60 (0.50, 5.16)

Table 4 (*continued*)

Cumulative REC (µg/m³-years)*	Ca	Co	OR** 95% CI
> 396	13	9	1.66 (0.68, 4.05)
p-trend			0.16

*Exposure categories as unexposed, > 0–50th percentile, > 50–90th percentile, > 90–95th percentile, and > 95th percentile.
**Adjusted for age, race, study, smoking status, and non-diesel exposed high-risk occupations.

reported work week in Spain was 48 hr/wk vs. 40 hr/wk in New England and longer duration worked in diesel-exposed jobs in Spain). Our data are consistent with a previous study in Montreal showing a similar prevalence of occupational diesel exposure in the general population as well as an increased risk for bladder cancer (Latifovic et al., 2015).

Analyses exploring the exposure-response relationship by lagging from 5 to 40 years showed a consistent increased risk at the top end of exposure, cumulative REC > 396.1 µg/m³-years, for all lag intervals. Our results also suggested a monotonic gradient in risk with cumulative REC lagged 40 years, but the numbers become limited, precluding us from drawing any firm conclusion regarding the shape of the exposure-response curve. A long latent period (up to 50 years) for bladder cancer has been previously observed in truck drivers (a major diesel-exposed occupation) in the National Bladder Cancer Study (Silverman et al., 1986). More studies are needed to better estimate the exposure-response relationship and the latent period for diesel-induced bladder cancer.

Our findings suggest that diesel is consistently associated with both low-grade tumors and in the development of muscle-invasive bladder cancer. Unfortunately, we do not have follow-up data to evaluate whether the group of low-grade tumors would have progressed to tumors with a worse prognosis. Similarly, we also do not have information on time to death among those initially diagnosed with muscle-invasive bladder cancer among patients with higher cumulative REC. Recently, we reported that a history of occupational diesel exhaust exposure was also associated with shorter time to recurrence among patients with muscle-invasive bladder cancer (Wilcox et al., 2016). Population-based estimates of incident bladder cancer indicate that 20–30% of bladder cancer is muscle-invasive and about 70–80% is non-muscle invasive. Further characterization of the tumors in these two case-control studies is underway to evaluate if diesel exhaust exposure is related to specific molecular subtypes or other tumor features (Choi et al., 2017).

Mechanistic evidence suggests that diesel exhaust may induce cancer in humans (IARC, 2014). Chromosomal damage, altered gene expression patterns, and inflammation have been found among people exposed to diesel exhaust (IARC, 2014). Urinary biomonitoring studies have demonstrated the presence of excreted polycyclic aromatic hydrocarbons and nitroarene metabolites, many of which are known to be genotoxic, after exposure to diesel exhaust (IARC, 2014; Seidel et al., 2002). Thus, direct contact with the bladder urothelium may be one pathway by which diesel exhaust initiates bladder carcinogenesis. Evidence from experimental studies also suggests that some components of diesel exhaust influence expression of genes related to critical cancer signaling pathways (IARC, 2014; Landvik et al., 2007; Yun et al., 2009).

Our study is one of the first to estimate UBC risk by quantitative estimates of lifetime occupational diesel exposure. The assessment of exposure was based on detailed subject-reported information on diesel-related work activities and intensity estimates derived from the published literature, reflecting an important advance in the characterization of diesel exhaust exposure compared with most previous case-control studies of UBC. This approach was also used in a study of occupational exposure to metalworking fluids in the NEBCS, revealing
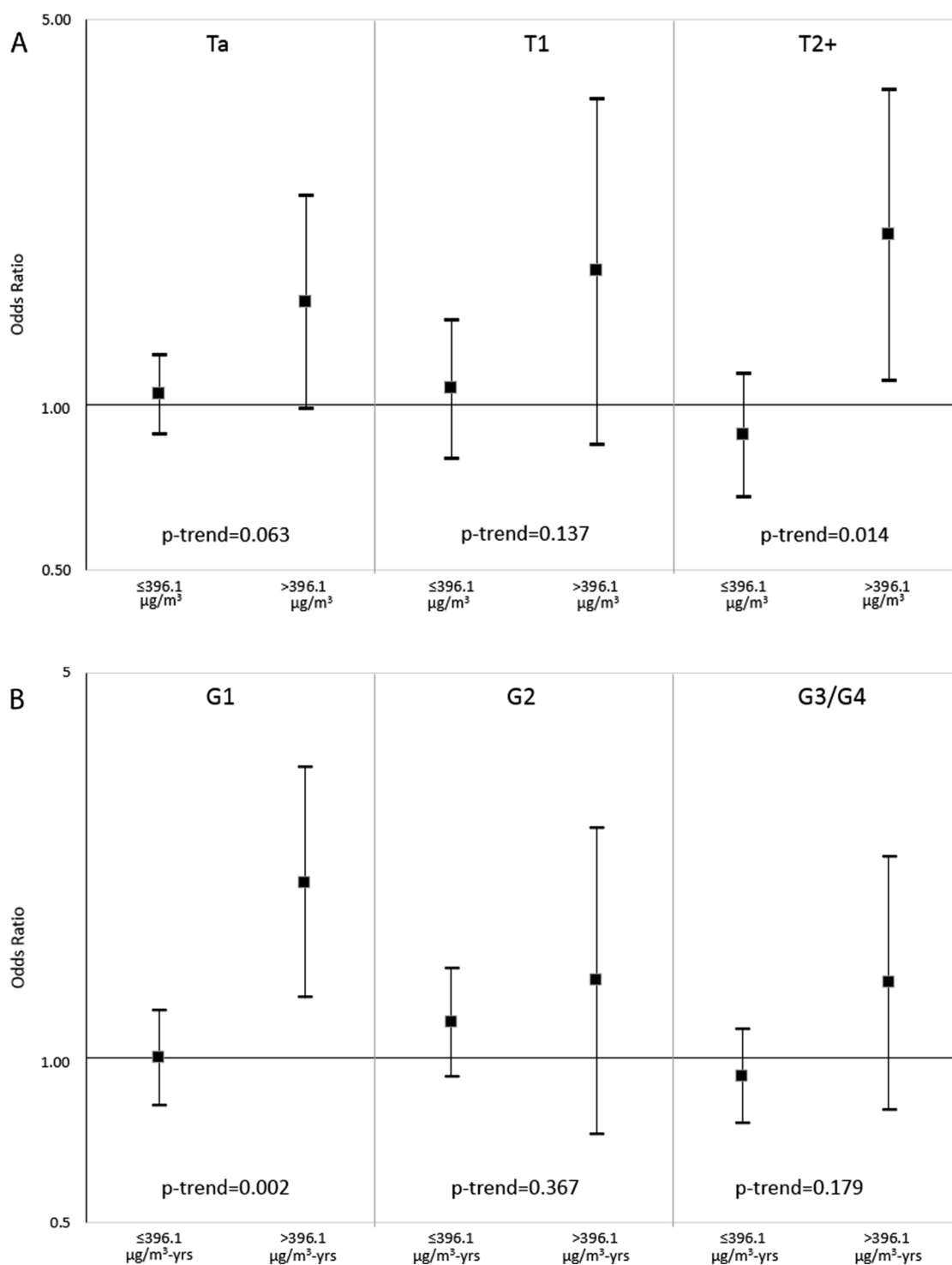
Fig. 1. Odds ratios from polytomous logistic regression for categories of cumulative REC (µg/m³-years), ≤ 396 µg/m³-years and > 396 µg/m³-years compared to the unexposed, and risk of UBC by pathologic stage (Panel A) and grade (Panel B). Models are adjusted for age, race, study, smoking status, and non-diesel exposed high-risk occupations. See also Supplemental Table A.3 for point estimates.

increased cancer risks similar to those observed in cohort studies (Silverman et al., 2018). Other strengths of our study include its large size (and consistency of observed associations in studies conducted in two different populations in different countries), as well as the ability to control for confounding from smoking and employment in other high-risk occupations. Further, the incorporation of pathology data provided the insight that the diesel exhaust-UBC relationship may be more pronounced in patients with later-stage tumors. This observation will help

guide future mechanistic work to better understand diesel-induced bladder carcinogenesis.

Some limitations, however, must be noted. Non-differential exposure misclassification likely occurred, probably leading to an attenuation of the observed associations and may have obscured effect at low levels of diesel-exposure (i.e., REC < 396 µg/m³). In addition, the published literature was insufficient to extract time trends in exposure intensity. Time trends in the probability of exposure were, however,

incorporated to take into account slight differences by country and regulations/technology that impacted the prevalence of diesel exposure. Interestingly, analyses by decade of first diesel-exposed job showed a statistically significant increased risk among men first exposed in the 1950s, when diesel equipment started becoming widespread. The observed greater risk for those first exposed in the 1950s may reflect secular differences in exposure by type or age of diesel engine, or alternatively, a long latent period for UBC. Despite the large size of these two case-control studies, the analysis was underpowered to detect a significant exposure-response; further exploration in other studies is warranted.

5. Conclusion

Our results provide further evidence that diesel exhaust exposure increases the risk of UBC in humans. The incorporation of quantitative historical information combined with pathologic characteristics of tumors and molecular epidemiology studies of exposed workers may provide evidence of biologic plausibility that this association is causal.

CRediT authorship contribution statement

Stella Koutros: Project administration, Formal analysis, Writing - original draft, Writing - review & editing, Data curation, Visualization. **Manolis Kogevinas:** Conceptualization, Investigation, Writing - review & editing. **Melissa C. Friesen:** Methodology, Writing - review & editing. **Patricia A. Stewart:** Methodology, Writing - review & editing. **Dalsu Baris:** Investigation, Writing - review & editing. **Margaret R. Karagas:** Investigation, Writing - review & editing. **Molly Schwenn:** Writing - review & editing, Investigation. **Alison Johnson:** Writing - review & editing, Investigation. **G.M. Monawar Hosain:** Writing - review & editing. **Consol Serra:** Writing - review & editing. **Adonina Tardon:** Writing - review & editing. **Alfredo Carrato:** Writing - review & editing. **Reina Garcia-Closas:** Writing - review & editing. **Lee E. Moore:** Investigation, Writing - review & editing, Resources. **Michael L. Nickerson:** Investigation, Writing - review & editing. **Stephen M. Hewitt:** Investigation, Writing - review & editing. **Petra Lenz:** Investigation, Writing - review & editing. **Alan R. Schned:** Investigation, Writing - review & editing. **Josep Lloreta:** Writing - review & editing. **Yves Allory:** Writing - review & editing. **Haoyu Zhang:** Methodology, Writing - review & editing. **Nilanjan Chatterjee:** Methodology, Writing - review & editing. **Montserrat Garcia-Closas:** Writing - review & editing. **Nathaniel Rothman:** Writing - review & editing. **Núria Malats:** Supervision, Project administration, Writing - review & editing, Investigation, Resources. **Debra T. Silverman:** Conceptualization, Supervision, Writing - original draft, Project administration, Writing - review & editing, Investigation, Resources.

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Declaration of Competing Interest

The authors declare no competing financial interests.

Appendix

(See).

Table A1
Job titles (in rank order) for most frequently reported occupations with diesel exhaust exposure.

Job titles
Truck drivers, tractor-trailer
Truck drivers, heavy
Grader, dozer, and scraper operators
Motor vehicle operators
Military occupations
Bus and truck engine, and diesel engine mechanics
Automobile mechanics
Timber cutting and related occupations
Garage and service station related occupations
Industrial truck and tractor equipment operators
Truck drivers, light

Table A2
Specific UBC tumor marker test for heterogeneity in cumulative REC exposure (linear trend) using a two-stage model.

Tumor Characteristic	Cumulative REC ($\mu g/m^3$-years)	
	Case-case OR* (95% CI)	p-value
P53 expression $^{\pm}$ (+/−)	1.07 (0.97, 1.19)	0.15
P53 Histoscore $^{\pm}$ (+/−)	1.04 (0.94, 1.15)	0.42
TP53 Mutation $^{\pm}$ (+/−)	0.96 (0.82, 1.13)	0.62
FGFR3 expression $^{\pm}$ (+/−)	1.00 (0.90, 1.12)	0.94
FGFR3 Mutation $^{\pm}$ (+/−)	1.08 (0.95, 1.23)	0.22

*Adjusted for age, race, study, smoking status, and non-diesel exposed high-risk occupations. $\pm$ Model includes noted tumor marker as well as stage and grade.

Table A3

Odds ratios for cumulative REC by tumor subtype from polytomous logistic regression presented in Fig. 1.

Stage	N Cases	N Controls	OR* (95% CI)
Ta			
Unexposed	464	857	1.0
≤396 µg/m^3-years	543	981	1.04 (0.89, 1.23)
> 396 µg/m^3-years	39	52	1.54 (0.98, 2.40)
p-trend			0.063
T1			
Unexposed	100	857	1.0
≤ 396 µg/m^3-years	132	981	1.07 (0.80, 1.43)
> 396 µg/m^3-years	10	52	1.75 (0.85, 3.61)
p-trend			0.137
(T2 +) Muscle-invasive bladder cancer			
Unexposed	161	857	1.0
≤ 396 µg/m^3-years	143	981	0.88 (0.68, 1.14)
> 396 µg/m^3-years	16	52	2.03 (1.11, 3.74)
p-trend			0.014
Grade			
G1			
Unexposed	253	857	1.0
≤396 µg/m^3-yrs	316	981	1.00 (0.81, 1.22)
> 396 µg/m^3-yrs	31	52	2.08 (1.28, 3.38)
p-trend			0.002
G2			
Unexposed	186	857	1.0
≤ 396 µg/m^3-years	218	981	1.16 (0.92, 1.46)
> 396 µg/m^3-years	13	52	1.37 (0.72, 2.62)
p-trend			0.367
G3/G4			
Unexposed	283	857	1.0
≤ 396 µg/m^3-years	281	981	0.93 (0.76, 1.13)
> 396 µg/m^3-years	21	52	1.37 (0.80, 2.33)
p-trend			0.179

OR$_{trend}$ for CIS = 0.97 (0.71, 1.34).

*Adjusted for age, race, study, smoking status, and non-diesel exposed high-risk occupations.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105346.

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