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A biological approach to characterizing exposure to metalworking fluids and risk of prostate cancer (United States)

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Abstract

Objective: Prostate cancer is hormone-related and chemicals that interfere with hormones may contribute to carcinogenesis. In a cohort of autoworkers we characterized exposure to metalworking fluids (MWF) into age windows with homogenous biological risk for prostate cancer, and examined exposure–response relationships using semi-parametric modeling.
Methods: Incident cases (n = 872) were identified via Michigan cancer registry from 1985 through 2000. Controls were selected using incidence-density sampling, 5:1 ratio. Using a hormonal-based model, exposure was accumulated in three windows: (1) late puberty, (2) adulthood, and (3) middle age. We used penalized splines to model risk as a smooth function of exposure, and controlled for race and calendar year of diagnosis in a Cox model.
Results: Risk of prostate cancer linearly increased with exposure to straight MWF in the first window, with a relative risk of 2.4 per 10 mg/m^3-years. Autoworkers exposed to MWF at a young age also had an increased risk associated with MWF exposure incurred later in life. For soluble MWF there was a slightly increased risk in the third window.
Conclusions: Exposure characterization based on a hormonal model identified heightened risk with early age of exposure to straight MWF. Results also support a long latency period for exposure related prostate cancer.

Introduction

Prostate cancer is a hormone-related cancer and thus shares at least one common mechanism of carcinogenesis with cancers of the breast, endometrium, ovary, testis, thyroid, and osteosarcoma. Hormones, both endogenous and exogenous, by driving cell proliferation, increase the number of cell divisions and the opportunity for random genetic errors [1]. Therefore, unlike 'classic' chemical carcinogenesis, the presence of a specific initiator may not be required, and DNA replication errors during cell divisions can create random mutations, which may give rise to a malignant cell.

A causal relationship between androgens and prostate cancer is biologically plausible because the vast majority of human prostate cancers are androgen sensitive, and men castrated prior to puberty are not known to develop prostate cancer [2, 3]. Epidemiological studies that have looked at the relationship between serum levels of androgens and prostate cancer have been, however, inconsistent. Only one study by Gann *et al.* [4] showed that men with higher levels of testosterone have higher risk of prostate cancer, while most studies have reported negative findings [2, 5–7]. In a meta-analysis, Eaton *et al.* [8] found that there were no large differences in circulating hormones between men who subsequently develop prostate cancer and those who remained free of disease. In all these studies, however, serum levels of testosterone were measured in the fifth or sixth decade of life and it is possible that male hormonal levels during the third or fourth decade of life may be

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more relevant to the development of prostate cancer since this cancer has a long latency period.

Men, unlike women, do not have well-established reproductive life events, such as menarche, menopause, and pregnancy, which have been related to risk factors for cancers of the breast and endometrium [9–11]. However, prostate epithelial cells undergo substantial proliferation and differentiation during three time intervals in a man's life: the prenatal period, puberty, and at approximately 50 years of age. It has been suggested that hormonal levels during these critical time periods may be relevant to the development of prostate cancer [6]. During puberty serum levels of testosterone increase and prostate cells begin to proliferate rapidly because of the enhanced production of dihydrotestosterone (DHT) in both stromal and epithelial cells of the prostate. With aging, serum levels of testosterone, free testosterone, and DHT decrease, while serum levels of estradiol and sex hormone binding globulin (SHBG) increase. These changes begin at approximately 50 years of age and coincide with the rise of prostate cancer incidence suggesting that a testosterone/ estrogen hormonal imbalance may be one of the factors that contributes to risk of prostate cancer [6, 12]. Other biological mechanisms, however, may contribute to this increased risk.

Taking advantage of these potential changes of hormonal levels in men's lifetimes we attempted to characterize exposure to metalworking fluids (MWF) in distinct periods of homogenous biological risk, or windows, with cut-points defined at ages where hormonal changes occur. MWF are complex mixtures of petroleum oils or synthetic lubricant products and a variety of additives, which are used to lubricate, cool, prevent rust, and carry away metal parts during two major types of operations, machining and grinding. They are grouped into three categories: 1) straight oils, which are undiluted mineral oils; 2) soluble fluids, which are water emulsions of mineral and fatty oils; and 3) synthetic fluids, which are aqueous solutions of organic compounds such as glycols or organic ethanolamines. Some of the components present in MWF, such as polyaromatic hydrocarbons (PAH), alkanolamines, and nitrosamines have been classified by IARC as possible human carcinogens [13, 14]. Therefore the process of carcinogenesis of MWF might be very complex, occurring via several mechanisms, such as disruption of endogenous hormones, action as exogenous hormones, or direct interaction with DNA to form DNA-adducts as in the case of PAH.

The objective of the current study was to explore risk of prostate cancer incidence in relation to MWF, taking account of the distinct time periods of different levels of biological risk in men's lifetimes. To achieve this goal, consecutive biological windows of exposure to MWF were defined by selecting age cut-points where hormonal changes occur, and penalized splines were used to smooth exposure–response and examine risk of prostate cancer incidence in relation to these exposure windows. We were also interested in examining whether subjects hired at younger ages had a different susceptibility to prostate cancer risk in relation to subsequent exposure to MWF, than those hired at older ages.

Methods

Subjects

This is a nested case–control study in a cohort of 31,648 hourly employed male members of the United Autoworkers (UAW) union. Eligible subjects worked at least three years prior to January 1, 1985 in three General Motors (GM) auto-manufacturing plants in Michigan and were still alive by this date (a subset of the UAW-GM cohort originally described by Eisen *et al.* [15]). Study subjects were hired between 1921 and 1981, and followed for cancer incidence from 1985 through 2000. Information on vital status was available for the original UAW-GM cohort from 1985 through 1994. A recent update of vital status from 1995 through 2000 was done for a 10% random sample of the UAW-GM cohort via National Death Index, for the purpose of another study using a case–cohort analysis [16]. We used this information to select a group of controls as described below.

For this study, incident cases of prostate cancer ($n = 872$) in the UAW-GM cohort were identified via Michigan cancer registry from 1985 through 2000 (vital status of these cases was also reported by the Registry until end of 2000). Eligible controls were selected using incidence density sampling from among co-workers who remained at risk, i.e. free of disease, by the age of diagnosis of the case (risk age), with a sampling ratio 5:1. Eligible controls for cases that were diagnosed from 1985 through 1994 were selected from the entire cohort. Controls for those cases diagnosed from 1995 until 2000 were selected from the 10% random sample of the UAW-GM cohort with updated vital information, in order to make sure that controls were still alive in the calendar year that they achieved risk age (same age of the case). (These controls were free of prostate cancer, since the entire UAW-GM cohort was sent to Michigan cancer registry to identify incident cases.) No other matching factors were used.

Exposure assessment

The detailed exposure assessment is described elsewhere [17]. Briefly, industrial hygiene measurements for MWF, as well as historical information on fluid type and specific operations were collected from plant records and corporate databases. The absolute levels of exposures were based on both personal breathing zone and general area samples collected by researchers [18]. A scale factor was calculated for each MWF type and operation (grinding and machining) based on the ratio of company measurements to those collected by research industrial hygienists in the 1980s. These scale factors were multiplied by study measurements to generate past exposure concentrations of MWF aerosol in each unique plant, department, and job [17]. For our study, MWF exposures for subjects that were still actively employed at the end of 1994 were updated up to 2000 assuming no changes in exposure levels during the last six-year period of additional follow-up.

Defining hormonal windows

Cumulative exposures (mg/m^3-years) to soluble, straight, and synthetic MWF were divided into three contiguous exposure windows defined by age, to capture periods of differences in susceptibility of prostate cells due to hormonal changes. Based on information in the literature, distinct periods for potential hormonal changes were defined as: (1) puberty, when serum levels of testosterone and DHT increase rapidly; (2) early until later adulthood, when serum testosterone levels remain stable; and (3) middle age, starting at 50 years old, when serum levels of testosterone decrease while serum levels of estradiol and SHBG increase. Based on this hormonal biological model, we defined three consecutive windows of exposure: (1) from the beginning of puberty through early adulthood (defined as approximately age 23); (2) early adulthood until middle age (defined as age 50); and (3) middle age until age at diagnosis (cases) or risk age (controls).

Considerable attention was directed towards the selection of age cut-offs for the three windows of exposure. Age of puberty was defined by pubertal stage development for genital and pubic hair [19, 20]. Based on the Third National Health and Nutrition Examination Survey (NHANES III), the age at stage 2 (onset of puberty) for boys is around ten years old for genital maturation and 12 years old for pubic hair [21]. However, NHANES reported a shift toward younger ages of achieving puberty in comparison with previous surveys, and therefore we posited an older age of puberty onset in our study, 13 years old, to account for birth cohort effects. Adulthood for males is achieved around 17–18 years old [19, 20]. We combined this fact with the empirical distribution of age at hire to help select a cut-off for early adulthood. We also examined how sensitive the dose–response slope in the first window (puberty until early adulthood) was to the selection of the cut-point. Finally, the cut-point at 50 years of age was based on hormonal changes that occur due to a serum testosterone/estrogen ratio imbalance.

Statistical analysis

We used penalized splines (psplines) in a Cox model to allow the relationships between exposure windows of each MWF type (e.g. straight) and the log relative risk of prostate cancer incidence to be a smooth function, and adjusted for cumulative exposure to other two MWF types (e.g. soluble and synthetic), race, and calendar year of diagnosis. We considered race a potential confounder since incidence rates of prostate cancer are higher among African-Americans compared to Caucasians, and MWF exposure in three plants varied by race. In relation to calendar year of diagnosis, incidence rates of prostate cancer have increased non-linearly from 1985 through 2000, while on the other hand MWF exposure levels have decreased during this follow-up time.

Penalized splines were implemented by fitting regression splines using penalized least squares, with a relatively large number of knots and a penalty put on coefficients of adjacent knots [22]. The S-PLUS function *pspline* used to fit psplines in Cox models has a default setting for number and placement location of knots – there are n knots equally spaced across the range of X (exposure), where $n = 2.5$ times the degrees of freedom (df) [23]. The optimal df for the psplines for each exposure window was in turn selected by assessing goodness of fit, as measured by Akaike Information Criterion (AIC) for the Cox model. The models included three psplines for the three exposure windows for a specific type of MWF, adjusting with linear terms for other MWF types in exposure windows, race, and calendar year of diagnosis. We performed this procedure separately for soluble, straight, and synthetic MWF. χ^2 tests for the linear and non-linear components of the *pspline* function (given in the output of the model) were used to determine whether a spline or a linear function was more appropriate for the variables that were smoothed.

Conditional logistic regression was used to examine linear relationships between prostate cancer incidence and exposure windows of MWF, using *proc phreg* in

SAS [24] to estimate relative risks and 95% confidence limits. Potential confounding was assessed for each exposure, fitting models with each main effect and then controlling for change in parameter estimates of exposure variables when other variables entered or left the models. To investigate the hypothesis that subjects exposed to MWF during puberty, might have a different susceptibility to MWF induced prostate cancer, we added interaction terms between early exposure and cumulative exposure in the two later windows. To assess the interaction, exposure in the first window was redefined as a dummy variable with two levels: 1.0 if a subject had been exposed to MWF in this window, and zero otherwise. Goodness of fit was assessed by AIC.

Results

There were 872 incident cases of prostate cancer in the cohort diagnosed from 1985 through 2000. On average, cases were 70 years old when they were diagnosed (Table 1). The percentage of African-Americans was higher among cases (27.3%) compared to controls (15.3%). Cases were hired on average two years later than controls, but the mean age of hire was similar, 30.2 *versus* 30.9 years old, respectively (Table 1). There was a significant nonlinear relationship between prostate cancer incidence and age of hire ($p = 0.03$); with highest risk for subjects hired at young ages, followed by a decreasing risk until age 35; after that age the curve became flat (Figure 1). These results suggested that exposure occurring at early ages of hire might be important in later risk of prostate cancer in life. There was also a positive correlation between age of hire and year of hire ($r = 0.53$, $p < 0.001$), indicating that subjects who were hired at young ages were more likely to be hired in the earlier calendar years, in the 1940s and 1950s, when MWF exposure levels were higher.

To select an age cut-off for end of puberty, we considered the potential for bias versus variance. To increase the stability of exposure–response estimates we want exposure to have a high variance within each window. The distribution of age at hire showed that 1%, 10%, and 25% of subjects were hired by age 18, 20, and 23 years, respectively. Although after onset of adulthood, we selected age 23 as the cut-point for exposure window because we wanted to have more exposed subjects and a wider range of exposure in the first window (puberty to early adulthood).

Table 2 gives the distribution of cumulative exposures to soluble, straight, and synthetic MWF in three windows: (1) puberty to age 23; (2) age 23–50; and (3)

Table 1. Characteristics of prostate cancer cases and controls in the cohort of autoworkers exposed to MWF

	Cases	Controls
n	872	4357
Age at diagnosis, (years); Mean (SD)	70.4 (8.9)	
Race; n (%)		
Caucasian	504 (57.8)	2793 (64.1)
African-American	238 (27.3)	668 (15.3)
Unknown	130 (14.9)	896 (20.6)
Plant they have worked; n (%)		
Plant 1 (Detroit)	407 (46.7)	1725 (39.6)
Plant 2 (Ypsilanti)	235 (26.9)	1588 (36.5)
Plant 3 (Saginaw)	230 (26.4)	1044 (23.9)
Year of Birth		
Mean (SD)	1923 (10.1)	1920 (9.8)
Range	1893–1949	1890–1949
Year of Hire		
Mean (SD)	1953 (11.6)	1951 (11.4)
Range	1925–1981	1921–1980
Age at Hire (years)		
Mean (SD)	30.2 (9.4)	30.9 (9.5)
Range	16.5–60.5	15.5–63.2
Calendar year of diagnosis (risk age)		
Mean (SD)	1993 (3.8)	1991(3.7)
Range	1985–2000	1985–2000
Duration of employment (years)		
Mean (SD)	23.2 (11.3)	20.9 (11.5)
Range	3.0–51.9	3.0–49.9
Vital Status as of 2000, n (%)		
Alive	531 (60.9)	2431 (56.3)
Dead	341 (39.1)	1748 (40.5)
Unknown	–	138 (3.2)

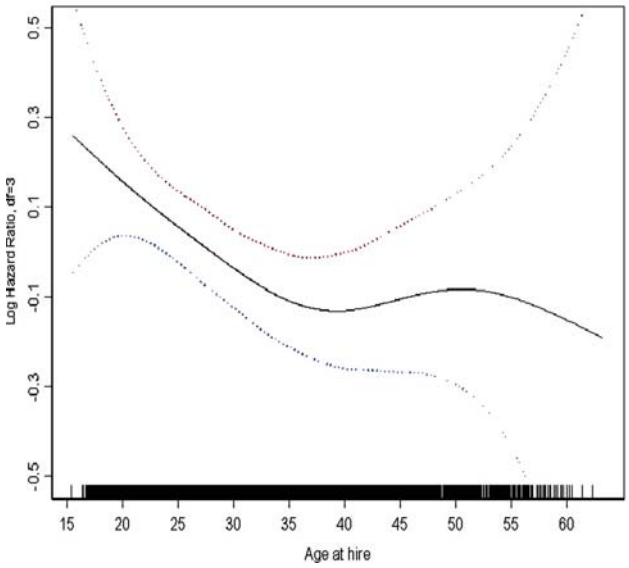

Fig. 1. Log relative risk of prostate cancer incidence in relation to age at hire using penalized splines (solid line). Rugs represent the distribution of exposure data; dotted lines represent confidence bands.

Table 2. Distribution of biological windows of exposure to three types of MWF among prostate cancer cases and controls

	Cases	Controls
Soluble MWF (mg/m^3-years)		
Puberty to age 23		
Mean (SD)	0.85 (2.62)	0.65 (2.75)
Range	0–27.72	0–45.50
Age 23–50		
Mean (SD)	12.64 (21.83)	11.76 (21.16)
Range	0–220.90	0–264.58
Greater than age 50		
Mean (SD)	4.81 (11.41)	4.60 (10.16)
Range	0–127.22	0–102.18
Straight MWF (mg/m^3-years)		
Puberty to age 23		
Mean (SD)	0.28 (1.74)	0.13 (0.98)
Range	0–26.28	0–26.16
Age 23–50		
Mean (SD)	3.14 (11.96)	2.28 (8.40)
Range	0–175.77	0–95.43
Greater than age 50		
Mean (SD)	1.16 (4.81)	0.92 (4.42)
Range	0–66.80	0–102.36
Synthetic MWF (mg/m^3-years)		
Puberty to age 23		
Mean (SD)	0.03 (0.29)	0.03 (0.48)
Range	0–4.77	0–25.47
Age 23–50		
Mean (SD)	0.57 (3.38)	0.48 (2.73)
Range	0–51.95	0–83.15
Greater than age 50		
Mean (SD)	0.23 (1.13)	0.28 (1.39)
Range	0–18.97	0–24.31

greater than age 50, for cases and controls, respectively. As seen for all three types of MWF the distributions were right-skewed and the mean was highest for the second window. In the earliest window (puberty to age 23) 19.3% of cases and 17.4% of controls had been exposed to soluble MWF, 9.4% of cases and 8.3% of controls had been exposed to straight MWF, and 2–3% of both groups had been exposed to synthetics; and the ranges of cumulative exposures were the smallest. Correlation coefficients between three consecutive windows of exposure for soluble MWF ranged from 0.15 to 0.33, however the first and the third windows of exposure were negatively correlated ($r = -0.21$). For straight and synthetic MWF, the ranges of correlation coefficients among three consecutive exposure windows were wider from 0.04 to 0.35, and 0.02 to 0.44, respectively.

Optimal degrees of freedom (df) for psplines were explored by minimizing the AIC value in Cox models, using smooth functions for each exposure window of one MWF type (e.g. straight), adjusting linearly for other exposure windows, cumulative exposure to the other two MWF types (soluble and synthetics), race, and calendar year of diagnosis. For soluble MWF the optimal df selected were 2.3, 1.2 and 1.4 for three windows: (1) puberty to age 23; (2) age 23–50; and (3) greater than age 50, respectively. For straight MWF, AIC was minimized for psplines with df of 1, 1.2, and 1.3, for each respective window, and for synthetic MWF the df selected were one for all three exposure windows. With so few degrees of freedom, psplines of three exposure windows for each MWF types indicated linear relationships with prostate cancer incidence. Therefore we fitted linear models.

Table 3 represents linear exposure-response models for prostate cancer in relation to three biological windows of exposure to soluble, straight, and synthetic MWF in three separate models. (Each model was adjusted for cumulative exposure to the other two types of MWF, race and calendar year of diagnosis). For cumulative exposure to soluble MWF in the first window, risk of prostate cancer increased modestly with a relative risk (RR) of 1.23 (95% CI 0.90, 1.62), however, with wide confidence intervals that included the null. In the second window, there was a flat exposure–response relationship, while in the last window, there was a slightly increased risk of prostate cancer with a RR of 1.11 (95% CI 0.99, 1.19) per 10 mg/m^3-years of cumulative exposure.

For straight MWF, there was a strong linear relationship between the first exposure window, puberty until age 23, and prostate cancer incidence with a RR of 2.38 (95% CI 1.31, 4.33) per 10 mg/m^3-years of cumulative exposure (Table 3). Evidence of a slightly increased risk was also seen in the second window, with a RR of 1.07 (95% CI 0.98, 1.17) per 10 mg/m^3-years, while there was a flat exposure–response relationship in the third window with wide confidence intervals that included the null. For synthetic MWF, there were null exposure–response relationships between either exposure window and prostate cancer (Table 3). Based on the estimates of these models, African-American autoworkers had an 85% increased risk of prostate cancer incidence compared to Caucasians (data not shown).

In order to examine how sensitive the exposure–response slope was in the first window to different age cut-points, we redefined the age of early adulthood as 20, 25, and 30 years old. We could not use age 18 as the cut-point for defining age of early adulthood, because only 1% of our subjects were hired before this age, with virtually no exposure to MWF. For each alternative age X, we accumulated exposure in the first window, age of puberty until age X, and fitted Cox models, adjusting for other two biological windows: age X until 50 years old,

Table 3. Exposure-response models for prostate cancer incidence in relation to biological windows of exposure to MWF among autoworkers[a]

MWF Type	Number of Exposed Subjects		RR	(95% CI)
	Cases	Controls		
Soluble MWF (mg/m^3-years)[b]				
Puberty to age 23	168	739	1.23	(0.90, 1.62)
Age 23–50	578	3076	1.01	(0.96, 1.05)
Greater than age 50	456	2116	1.11	(0.99, 1.19)
Straight MWF (mg/m^3-years)[b]				
Puberty to age 23	82	349	2.38	(1.31, 4.33)
Age 23–50	340	1800	1.07	(0.98, 1.17)
Greater than age 50	225	1041	1.04	(0.87, 1.24)
Synthetic MWF (mg/m^3-years)[b]				
Puberty to age 23	21	91	0.86	(0.18, 4.14)
Age 23–50	131	802	1.19	(0.91, 1.56)
Greater than age 50	141	709	0.75	(0.35, 1.57)

[a] Each model is adjusted for cumulative exposure to other two types of MWF, race and calendar year of diagnosis.
[b] Cumulative exposure was used as continuous; relative risk expressed as change in RR per 10 mg/m^3 years unit increase of exposure

and age 50 until risk age, as well as for race, and calendar year of diagnosis. Since results of exposure–response relationships were strongest for straight MWF we restricted the sensitivity analysis to this fluid type. Figure 2 shows the psplines of log relative risk of prostate cancer incidence in relation to cumulative exposure to straight MWF in the first window, with four alternative ages cut-offs. As can be seen, the slope is initially steepest when using age 20 as the cut-off, and became less steep with increasing age. Results of this sensitivity analysis suggest that exposure to straight MWF occurring at young ages–when prostate tissue might be more susceptible – may have an important role in risk of prostate cancer later in life.

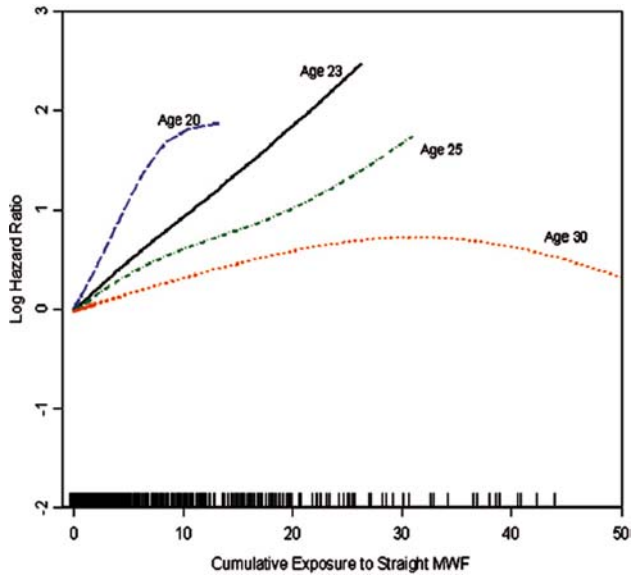

Fig. 2. Sensitivity of the exposure–response curve in the first biological window due to different age cut points. Lines represent the curves of log relative risk of prostate cancer incidence in relation to exposure to straight MWF from puberty until age of early adulthood, with four different age cut-points of 20, 23, 25 and 30 years. Rug represents the distribution of exposure data.

To investigate the hypothesis that subjects exposed to MWF before age 23 might experience a higher susceptibility to MWF induced prostate cancer, we added two interaction terms between early exposure (in the first window) and cumulative exposures in the later windows. To assess the interaction, exposure in the first window was redefined as a dummy variable with two levels: 1.0 if a subject had been exposed to MWF by age 23 (i.e. in the first window), and zero otherwise. (The definition for the main effect of this exposure window remained the same, continuous.) For soluble MWF, there was no effect modification; i.e. subjects exposed before age 23 did not have higher risk of prostate cancer due to exposure incurred in the later windows compared to those subjects exposed to MWF after age 23 (data not shown). In relation to straight MWF, results of interactions are presented in models 2 and 3 of Table 4. As seen from model 2, there was no effect modification for the second exposure window of straight MWF between subjects who were exposed to straight MWF before, or after age 23 years. By contrast, there was a strong interaction between cumulative exposure to straight MWF in the third window, and ever having been exposed to straight MWF before age 23, i.e. in the first window, with a RR of 6.46 (95%CI 1.12, 37.26) per 10 mg/m^3 years (model 3, Table 4). Results suggest that subjects exposed to straight MWF by age 23 have a higher risk of exposure-related prostate cancer in the third exposure window, after age 50, compared to those who were not exposed at young ages.

Table 4. Susceptibility to prostate cancer in relation to biological windows of exposure to straight MWF[a]

	Model 1		Model 2		Model 3	
	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)
Straight MWF (mg/m^3-years)[b]						
Puberty to age 23 (first)	2.38	(1.31, 4.33)	2.24	(1.18, 4.26)	2.27	(1.24, 4.16)
Age 23–50 (second)	1.07	(0.98, 1.17)	1.06	(0.97, 1.17)	1.05	(0.96, 1.15)
Greater than age 50 (third)	1.04	(0.87, 1.24)	1.04	(0.85, 1.31)	1.04	(0.87, 1.24)
Interaction of second and first[c]			1.06	(0.85, 1.31)		
Interaction of third and first[d]					6.46	(1.12, 37.26)
Model Fit[e]						
AIC	2705.99		2707.74		2703.73	

[a] Each model is adjusted for cumulative exposure to other two types of MWF, race and calendar year of diagnosis.

[b] Cumulative exposure was used as a continuous variable; relative risk expressed as change in RR per 10 mg/m^3 years unit increase of exposure.

[c,d] These interaction terms were created between cumulative exposure to straight MWF, used as a continuous variable, in the second or third window, respectively, and a dummy variable defined as: 1 if ever exposed to straight MWF before age 23, i.e. in the first window, 0 otherwise.

[e] AIC value for the model without covariates was 3031.32.

Discussion

In this paper we characterized exposure to three types of MWF: straight, soluble, and synthetic, into time periods with hypothesized homogenous biological risk for prostate cancer, and examined exposure–response relationships using penalized splines in Cox models. Results showed that risk of prostate cancer increased linearly with exposure to straight MWF from puberty to early adulthood, with a relative risk (RR) of 2.4 per 10 mg/m^3-years of cumulative exposure. Evidence of a slight increase in risk was seen in the second window, age 23–50, with a RR of 1.1 per 10 mg/m^3-years, and no exposure–response relationship in the third window. In relation to water-based fluids the evidence for an association was weaker. There was a slight increase in the relative risk of prostate cancer with exposure to soluble MWF in the third window, after age 50, with a RR of 1.1 per 10 mg/m^3-years, and no exposure-response relationships for the other two windows of soluble MWF exposure. For synthetic MWF there were no exposure–response relationships in either window.

Sensitivity analysis showed that the exposure–response slope in the first window of straight MWF exposure was steeper, the younger the age cut-off for early adulthood was defined. This finding suggested that MWF exposures that occur at very young ages of hire, when prostate tissue is more susceptible to external exposures because of enhanced proliferation and differentiation due to hormonal changes, might have an important role in risk of prostate cancer later in life. In addition to the previous findings, results suggested that subjects exposed to straight MWF at very young ages, from puberty until early adulthood, also had a higher susceptibility to prostate cancer risk from subsequent exposure to MWF, with a relative risk of 6.5 due to exposure incurred later in life during middle age.

The results of our study suggest that straight MWF exposure occurring from puberty until early adulthood is strongly associated with risk of prostate cancer later in life and shed light on a potential interaction with sex hormones. Very few studies have evaluated the role of sex hormones during puberty and subsequent prostate cancer risk. Ross *et al.* [25] in their study found that young African-American men had 15% and 13% higher testosterone and free testosterone levels, respectively, compared to their Caucasians counterparts; a finding that could explain in part higher incidence rates of prostate cancer among African-American males. Results of our study also support an early effect of MWF, however, since we did not have any information on sex hormone levels during life for our subjects we could not assess directly the interaction of MWF with these hormones. We characterized MWF exposure based on certain assumptions about the typical biologically-defined time periods, in which serum levels of both androgens, estrogens, and SHBG are relatively constant within a time period, but they differ from one period to another. These changes in time periods make the prostate cells more susceptible to external exposures such as MWF.

In our approach cumulative exposure was divided into periods based on a hormonal model and results were strongest for straight MWF, showing an early exposure effect. Other potential explanation for this early exposure effect could be: (1) a long latency period for exposure-related prostate cancer, as well as (2) higher concentrations of MWF in earlier calendar periods of time in the 50s and 60s when also polyaromatic hydrocarbons (PAH) content was higher. In

another analysis of the same data, when cumulative exposure to MWF was divided into contiguous windows based on cancer latency, risk of prostate cancer incidence was increased with exposure to soluble and straight MWF ≥ 25 years before risk age, but not with exposure in the more recent window, <25 years [26]. For straight MWF there was a linear relationship with prostate cancer incidence, with a RR of 1.12 per 10 mg/m^3-years of cumulative exposure [26]. In metalworking industries, MWF concentrations have decreased substantially over time with lower levels in the 80s. In addition PAH concentrations in MWF (present in varying degrees in straight and soluble MWF but not synthetic MWF) were higher before the 1970s and have decreased over time due to better refining of mineral oils [27]. Besides PAH, other chemical compounds that are formed during chlorination of base oils such as chlorinated paraffin, and naphthenic compounds have shown carcinogenic effect in animal studies, and during MWF use these chlorinated compounds may react to form other potentially carcinogenic substances such as dioxins [27, 28].

In our study we did not have information on other risk factors for prostate cancer such as family history, or diet. The strongest unmeasured risk factor is family history of prostate cancer, which is associated with both increased risk and earlier onset of disease in first-degree relatives, and the relative risks estimates from different studies range from 1.7 to 3.7 [29]. Other risk factors such as diet, fat intake, alcohol consumption, smoking, and vitamins, have been investigated but the evidence is not clear. Vitamin D and calcium intake, especially from dairy products, have been associated with increased risk of prostate cancer [30]. There is no clear association between cigarette smoking and prostate cancer [31], and only very heavy alcohol consumption (7 or more drinks per day) has been associated with an excess risk of prostate cancer [32, 33]. Some of the unmeasured lifestyle risk factors might have differed among autoworkers by exposure levels, and possible confounding could partly explain the attenuated exposure–response relationships. However, Kriebel *et al.* [34], using data from the UAW-GM cohort, have recently shown that in retrospective cohort studies, where information on potential confounding factors might be missing, it is unlikely that either systematic or chance differences in lifestyle factors would cause as much as 20% change in the relative risk estimates of the exposure–disease relationships.

In conclusion, results of this study provide evidence that early exposure to straight MWF, and neither soluble nor synthetic fluids, was strongly associated with prostate cancer risk among autoworkers. In the absence of clearly observable reproductive events for men, such as those identified as risk factors for female hormonal cancers of the breast and uterus, these findings also suggest that the approach based on measuring exposure in hormonally defined windows may prove useful for other studies of occupational and environmental causes of prostate cancer.

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