Polycyclic Aromatic Hydrocarbon Exposure, Urinary Mutagenicity, and DNA Adducts in Rubber Manufacturing Workers

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Abstract

Objectives: **Several studies have suggested that genotoxic risks might still be present in the contemporary rubber manufacturing industry. Previously, we observed elevated levels of urinary mutagenicity and bladder DNA adducts in rubber workers. Presently, we investigated whether DNA adducts in peripheral blood mononuclear cells (PBMC) and/or urothelial cells may be caused by polycyclic aromatic hydrocarbons or other genotoxic compounds.**

Methods: **Spot urine samples from 116 rubber manufacturing workers were collected on Sunday and during the workweek (post-shift) to determine 1-hydroxypyrene and mutagenicity levels. For 52 nonsmokers, urothelial cell DNA adducts and PBMC DNA adducts were measured additionally.**

Results: **Urinary 1-hydroxypyrene levels were significantly higher in workweek samples compared with Sunday (P = 0.0001). This increase was not uniform across tasks and only reached statistical significance for the curing department (+99%; P = 0.003). Weekday urinary mutagenicity was significantly increased for mixing (+56%) and curing (+21%) workers when compared with that for Sunday. Total urothelial cell DNA adducts were related to urinary 1-hydroxypyrene (P = 0.021) and mutagenicity (P = 0.027). No significant relationship was found between the adduct levels in PBMC and urothelial cells or between the former and urinary 1-hydroxypyrene or mutagenicity.**

Conclusions: **Workers in the compounding, mixing, and curing departments were at highest genotoxic risk among rubber manufacturing workers. Increased levels of urinary 1-hydroxypyrene, mutagenicity, and urothelial cell DNA adducts were found in these workers. Urothelial cell and PBMC DNA adducts were not related, hinting possibly to the presence of specific bladder carcinogens in the rubber manufacturing industry.** (Cancer Epidemiol Biomarkers Prev 2008;17(6):1452–9)

Introduction

Employment in the rubber manufacturing industry has been identified as high-risk occupation for several cancer sites, in particular, the lungs, urinary bladder, and larynx, as well as leukemia (1). Relating the observed risks within the industry to specific exposures has been challenging because of the complex nature of the exposure that can include several potential genotoxic and carcinogenic agents. Exposures to aromatic amines, carbon black, polycyclic aromatic hydrocarbons (PAH), nitrosamines, and solvents have been reported (1, 2). Airborne particulate levels seem to be higher in the beginning of the production process, that is, during handling weighing and mixing of raw materials, and to decrease further down the production process (3). Solvents are mainly used during the assembly of rubber goods (4). Curing generates rubber fumes and gases that can contain PAHs and nitrosamines. Finished products can still off-gas contaminants during inspection, finishing, and storage. A downward trend has been observed in the inhalation of dust, rubber fumes, and nitrosamines, and dermal exposure levels in the 1990s in the rubber industry (3, 5). Nevertheless, elevated cancer risks in this occupational group have continued to be reported after the evaluation of the IARC in 1982 (2). However, it is unclear if these observed risks are representative of current exposure circumstances in the rubber industry. Studies focusing on genotoxic exposures and early effects could therefore provide some insight on current genotoxic risks in this particular industry.

We have previously reported that rubber workers excrete higher levels of mutagens in weekday urine compared with Sunday urine samples (6). In addition, we observed elevated bladder DNA adduct levels in previously identified high-risk production functions of compounding, mixing, and curing compared with other departments (7). These results indicated the presence of genotoxic exposures in the contemporary rubber manufacturing industry in the Netherlands. However, it remained unclear to which agent or group of agents these elevated mutagenicity and adduct levels could be

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attributed. Interestingly, in an exploratory analysis, an association between PAH exposure and a specific bladder DNA adduct was observed (8). This suggested a possible role of PAH exposure in the observed genotoxic risk. Major PAH sources in the rubber manufacturing industry are the extender oils, which could make up as much as 20% of the total wet weight of rubber products, and possibly carbon black, which also is a major component. PAHs may also be formed by the incomplete combustion or pyrolysis of organic material during curing (8).

To follow-up these findings, we analyzed urinary 1-hydroxypyrene levels among the same group of rubber manufacturing workers from the industry-wide cross-sectional survey as analyzed in the earlier reports. Our current focus was on the possible relationship among PAH exposure, urinary mutagenicity, and bladder DNA adducts. In addition, we measured bulky DNA adducts in peripheral blood mononuclear cells (PBMC). Comparing DNA adduct levels in PBMCs with levels in the target tissue cells (that is, the urinary bladder) could provide information on whether there are genotoxic agents present that act as general carcinogens or as specific bladder carcinogens.

Materials and Methods

Cross-sectional Study. The field phase of the study was conducted in 1997 among subjects working in the Dutch rubber manufacturing industry (9). Subjects (N = 225) were employed in nine companies (three rubber tire/belts, five general rubber goods, and one retreading company) and were randomly selected based on their production function (that is, compounding and mixing, pretreating, molding, curing, finishing, shipping, engineering service, and laboratory). Details of this selection are described elsewhere (10). Fieldwork was carried out within 1 week per company and included a medical survey, ambient and personal exposure measurements, and collection of biological samples. Urinary analyses were done only in a subpopulation consisting of ~50% random sample stratified by company and department from the initial population. The selection contained 116 male subjects with a mean age of 38.5 years, both smokers (n = 45) and nonsmokers (n = 71), and covered all factories and departments (11). Results reported here are restricted to this subpopulation. The study was approved by the institutional review board. Participation was voluntary, and written informed consent was obtained from participants.

Urine and Blood Collection. Spot urine samples were collected on Sunday and during the workweek, on Tuesday, Wednesday, and Thursday at ~4 p.m., and they were stored at −20°C till analyses. Twenty-four-hour urine samples were collected from Monday (after first morning void) to Tuesday (including first morning void). These samples were kept cold (±4°C) during collection and with 20% glycerol (volume for volume) to minimize cell loss due to lysis after freezing (−20°C). Blood samples were collected at the end of the workweek (Thursday or Friday) using cell preparation tubes (BD Vacutainer).

Urinary 1-Hydroxypyrene. Spot urine samples were analyzed for 1-hydroxypyrene according to the method developed by Jongeneelen et al. (12). In brief, the urine was acidified and the glucuronic acid and sulfate were enzymatically removed. The samples were purified using solid phase extraction and analyzed by liquid chromatography and fluorescence detection. Creatinine levels were used to estimate urinary dilution using a colometric test, based on the Jaffé reaction between creatinine and sodium picrate, or analyzed according to an enzymatic method described by Mazzachi et al. (13). Urinary 1-hydroxypyrene was expressed as micromoles per mole of creatinine.

Urinary Cotinine. Mainstream and environmental tobacco smoke intake was evaluated by assessing cotinine levels in Sunday and weekday urine samples. Urinary cotinine was quantified by high-performance liquid chromatography according to the method of Barlow et al. (14), using the modifications described by Parviainen and Barlow (15). Cotinine levels were expressed as microgram per gram of creatinine.

Urinary Mutagenicity. Urinary mutagenicity was determined as described earlier (6). Volumes corresponding to 0.5-mmol creatinine of the Wednesday (10-510 mL) and Thursday (35-610 mL) spot urine samples were pooled for each subject for mutagenicity analysis, using the *Salmonella typhimurium* strain YG1041 with an S9 mix from Aroclor 1254–induced rat livers (16, 17). Urinary mutagenicity levels were expressed as revertants per gram of creatinine. In some analyses, cotinine-corrected urinary mutagenicity levels were used. This correction was necessary because smoking habits differed between weekend and workweek within the same individual (6). The cotinine-corrected Sunday and pooled weekday urinary mutagenicity levels were calculated based on the algorithms for the relation between urinary cotinine and urinary mutagenicity levels as described previously within this population (6).

Urothelial Cell DNA Adducts. Sixty nonsmokers were randomly selected from the subpopulation. DNA ($>20 \times 10^7$ nucleotides) was successfully isolated from exfoliated urothelial cells of 52 subjects as described by Vermeulen et al. (7). ^{32}P-postlabeling was done under conditions of excess radiolabeled ATP. The first spot detected was assigned number 1, and each spot detected in the same position was thus labeled. Other spots detected in different positions in the chromatogram were numbered consecutively according to each new appearance on the plates. Carcinogen DNA adducts levels were calculated by determining the relative adduct labeling (RAL), which was defined as counts per minute in adduct spots per adjusted counts per minute in normal (unmodified) nucleotides. In the absence of a particular adduct spot in the individual map or a level of $<1 \times 10^7$ RAL, the DNA adduct level was designated at 50% of this level, resulting in 0.5×10^7 RAL.

PBMC DNA Adducts. DNA adduct analyses were done on blood samples of the 52 nonsmokers selected for urothelial cell DNA adduct analyses. Four subjects had refused blood collection, resulting in 48 DNA adduct analyses. PBMCs were isolated using CPT vacutainer tubes with a sodium-heparin anticoagulant with a Ficoll gradient and a polyester gel barrier. DNA was extracted from the PBMCs using the chloroform–isoamyl alcohol method (18). DNA samples were analyzed using the

^{32}P-postlabeling technique with nuclease P1 enrichment (19). DNA was hydrolyzed by incubation for 3 h at 37°C using 2.5 units of micrococcal endonuclease (Sigma Chemical Co.) and 0.25 μg (4 milliunits) of calf spleen phosphodiesterase (Sigma). Samples were again incubated for 40 min at 37°C to enhance the sensitivity of the assay after nuclease P1 (Sigma) was added (0.75 units) to the digested DNA. Adducted nucleotides were labeled by adding 50 μCi per sample of ^{32}P-ATP (Perkin-Elmer) and incubation with T4 polynucleotide kinase (5 units/μL; GE Healthcare) for 40 min at 37°C. Two microliters of potato apyrase (Sigma) were added to the mixture and incubated for 40 min at 37°C to hydrolyze any unreacted ATP.

Eighty percent of the sample volume was spotted on a polyethyleneimine-cellulose plate (10 × 10 cm; Alltech), and adducts were mapped with a three-directional TLC using elutions of (*a*) 0.65 mol/L of sodium phosphate (pH 6.0; Fisher); (*b*) 3.6 mol/L of lithium formate and 8.5 mol/L of urea (pH 3.5; Fisher); and (*c*) 0.8 mol/L of lithium chloride, 0.5 mol/L of Tris-HCl, and 8.5 mol/L of urea (pH 8.0; Fisher). Plates were developed in 1.5 mol/L sodium phosphate (pH 6). DNA adducts were visualized by autoradiography and quantified using Cerenkov counting. Plates were placed in autoradiography cassettes (Fisher) containing film (IsoBioexpress) and intensifying screens (Fisher), and exposed at −80°C for 48 h. Spots were excised from the plates and counted in plastic scintillation vials (Fisher) containing 5 mL of 70% ethanol. Appropriate blank areas of the chromatogram were also excised to provide a background adjustment. The first spot detected was assigned as letter A, and each spot detected in the same position was equally labeled. Other spots detected in different positions in the chromatogram were assigned alphabetically according to each new appearance on the plates.

Calculation for RAL was done using the following formula (18):

$$\mathrm{RAL} = \frac{\text{cpm in adducted nucleotides} \times \mu g\ DNA \text{ in sample}}{3 \times 10^6\ \text{cpm/pmol(ATP)} \times 3{,}240\ \text{pmol dNp/}\mu g\ DNA}$$

where cpm is counts per minute and dNp is the number of deoxynucleotide phosphates. Samples varied in their DNA concentration in the range of 0.1 to 1.5 μg/μL with 4 μL of DNA solution being used in every case. In the absence of a particular adduct spot in the individual map or a level of $<1 \times 10^7$ RAL, the DNA adduct level was designated at 50% of this level, resulting in 0.5×10^7 RAL.

Acetylation Status. Acetylation status was previously found to influence urinary mutagenicity and urothelial cell DNA adducts levels (7, 19). Acetylation status was determined by measuring caffeine and its metabolites as described by Grant et al. (20). Designation of the acetylator (NAT2) phenotype was based on the ratio between 5-acetylamino-6-formylamino-3-methyluracil and 1-methylxanthine (1×). Results showed a clear bimodality of *N*-acetylation capacity with a ratio cut point for fast- versus slow-acetylation status of 1.7, resulting in 61% slow and 39% fast acetylators.

Personal Exposure Measurements. Personal inhalable particulate exposure was measured on the days of urine collection (Tuesday to Thursday) by means of a PAS6 sampler in the breathing zone of the worker (21). On the same days, personal dermal exposure to cyclohexane soluble matter was measured with a dermal pad sampler worn at the lower part of the wrist of the hand of preference (3). The cyclohexane soluble matter contents of inhalable (e.g., rubber fumes) and dermal exposure samples were determined by means of NIOSH PCAM 217 method (22).

Statistical Analyses. Urinary 1-hydroxypyrene and mutagenicity levels are summarized as arithmetic means, geometric means, and geometric SDs. These data were log normally distributed, and therefore, natural logarithms were used in the statistical analyses. Summary information for the adduct data is given as arithmetic mean, mean SD, and percentage of samples with detectable adduct levels ($\geq 1 \times 10^7$ RAL).

Correlations between biomarker levels by day of urine collection were calculated using Pearson's correlation statistic. Differences in urinary biomarker levels between Sunday (background) and mean individual weekday level were tested for statistical significance by paired *t* tests. The median of the individual effect of the level during workweek compared with Sunday was calculated as well. Multivariate ANOVA was applied to study the effect of factory and department on urinary 1-hydroxypyrene, mutagenicity, and DNA adduct levels using mixed-effect models and general linear models. Factory and department were included as fixed effects in these models, whereas worker was included for 1-hydroxypyrene as a random effect in the mixed-effect models to account for repeated measures on the same person. These models were corrected for individual biomarker background levels and smoking by adding the Sunday urinary biomarker level and weekday urinary cotinine [to correct for (residual) tobacco smoke exposure] as additional fixed effects in the model.

Logistic regression analyses were done to explore the possible relations between the adduct levels in urothelial and peripheral blood cells and the two urinary biomarkers. Linear least square regression analyses were carried out to describe the association between urinary mutagenicity and 1-hydroxypyrene levels. In these analyses, the log of the averages (arithmetic mean) of the individual urinary biomarker levels was used. These models were adjusted for urinary cotinine and acetylation status (NAT2 phenotype; slow or fast acetylator). The latter adjustment by NAT2 phenotype as main effect was done as in previous analyses wherein an association among NAT2 phenotype, urinary mutagenicity, and urothelial cell DNA adducts was observed. All analyses were done using the SAS version 9.1 software (SAS Institute, Inc.).

Results

A valid Sunday and weekday urine mutagenicity measurement was obtained from 104 of 116 subjects (90%). Due to the limited amounts of urine available for the 1-hydroxypyrene measurements, 90 subjects (78%) had a valid Sunday measure of urinary 1-hydroxypyrene whereas 115 (99%) subjects had at least 1 valid weekday urine sample. Of the selected nonsmokers ($n = 60$) for the urothelial cell DNA adduct analyses, 52 subjects (87%)

had sufficient amounts of DNA for analyses. For 48 (80%) of these workers, DNA from PBMCs could be analyzed for bulky DNA adducts. In all situations, missing observations were randomly distributed across factories and departments.

Table 1 presents the influence of factory and department on the internal exposure and early effect markers. Urinary 1-hydroxypyrene and urothelial cell DNA adducts were highly influenced by the department where the subject worked and, to a lesser extent, by the specific factory. However, the effect of department was heterogeneous between factories as indicated by the significant interaction term between department and factory. Department was borderline statistically significantly related to urinary mutagenicity. Department and factory did not influence PBMC DNA adduct levels.

As shown in Fig. 1, urinary 1-hydroxypyrene levels were slightly but significantly increased during the workweek compared with Sunday levels (overall $P = 0.0001$). Nonsmokers had a geometric mean of 0.12 μmol/mol creatinine on Sunday and 0.17 to 0.20 μmol/mol creatinine during the workweek, whereas smokers had a geometric mean of 0.26 μmol/mol creatinine on Sunday and 0.29 to 0.35 μmol/mol creatinine during the workweek. Individual urinary 1-hydroxypyrene levels were strongly correlated between weekdays (r = 0.78-0.84; $P < 0.0001$), whereas the correlation between Sunday and weekday 1-hydroxypyrene levels was weaker (r = 0.40-0.41; $P = 0.0002$). Increases in individual weekday levels were highest for the molding, curing, and finishing departments. However, statistical significance was only reached for curing ($P = 0.003$), wherein the workers also showed the highest weekday 1-hydroxypyrene levels with a geometric mean of 0.31 μmol/mol creatinine (Table 2).

Analyses restricted to nonsmokers showed essentially the same results, except for molding, wherein the difference reached statistical significance ($P = 0.008$; results not presented). Overall, mutagenicity in weekday urines was higher compared with the Sunday samples (Table 2). Statistically significant increases were observed for compounding and mixing (+56%) and curing (+21%), whereas pretreating (+22%) and engineering (+68%) showed borderline significant increases. Decreases in urinary mutagenicity, although not significant, were seen in the molding and finishing departments, and laboratory. When comparing the two internal exposure markers (that is, urinary 1-hydroxypyrene and mutagenicity) with the external exposure data [inhalable dust, rubber fumes (cyclohexane soluble matter), and dermal cyclohexane soluble matter], a positive relationship seemed to exist between urinary 1-hydroxypyrene levels and both inhalable (rubber fumes) and dermal cyclohexane soluble matter exposure. Urinary mutagenicity, on the other hand, seemed to be more associated with inhalable dust exposure (Table 3).

Eleven possible DNA adducts were observed in the urothelial cell analyses, whereas in the PBMCs, seven possible adducts were found. Repeated analyses revealed that only four specific DNA adducts were reliably detected in both cell types. Table 4 shows the mean relative adduct levels of these four adducts. DNA adducts 1 and 3 were the dominant adducts found in exfoliated bladder cells. In PBMCs, adducts B and D were most frequently observed. There was no relationship between urothelial cell and PBMC DNA adducts for an individual. Total urothelial cell DNA adducts was significantly related to urinary 1-hydroxypyrene ($P = 0.035$) and mutagenicity ($P = 0.027$), as shown in Table 5. For 1-hydroxypyrene, this seemed to be mostly driven by adduct 3, whereas mutagenicity was more strongly related to adduct 1. Stratified analyses by acetylation status indicated that the association between mutagenicity and urothelial cell DNA adducts was predominantly present among the slow acetylators ($\beta = 1.18$ with $P = 0.042$ versus $\beta = 0.50$ with $P = 0.251$ for fast acetylators; P for interaction = 0.022). Blood cell DNA adducts were not correlated to any of the internal or external exposure markers.

Discussion

We evaluated relationships among external exposure, urinary 1-hydroxypyrene and mutagenicity as internal exposure levels, and DNA adducts as early biological effect markers in a population of Dutch rubber manufacturing workers. 1-Hydroxypyrene levels were significantly elevated during the workweek compared with Sunday, indicating occupational PAH exposure in the contemporary rubber manufacturing industry. The increase

Table 1. *P* values for the type 3 tests of the fixed effects of factory and department on exposure and early effect markers

Model*	Fixed effect	*P*			
		1-Hydroxypyrene †,‡	Mutagenicity †,§	Bladder adducts§	PBMC adducts§
1	Factory	0.1743	0.6874	0.6908	0.6047
2	Department	0.0191	0.0582	0.0001	0.9136
3	Factory	0.1798	0.8036	0.8879	0.4748
	Department	0.0250	0.1057	0.0016	0.7280
4	Factory	0.1059	0.8005	0.0890	0.8512
	Department	0.0104	0.3360	<0.0001	0.7704
	Factory × department	0.0038	0.9302	0.0029	0.9141

*Description of models: 1, univariate model with factory as fixed effect; 2, univariate model with department as fixed effect; 3, multivariate model with factory and department as fixed effects; 4, multivariate model with factory and department as fixed effects and the interaction between factory and department.

†Urinary 1-hydroxypyrene and mutagenicity are corrected for individual Sunday biomarker and cotinine level.

‡Mixed-effect models.

§General linear models.

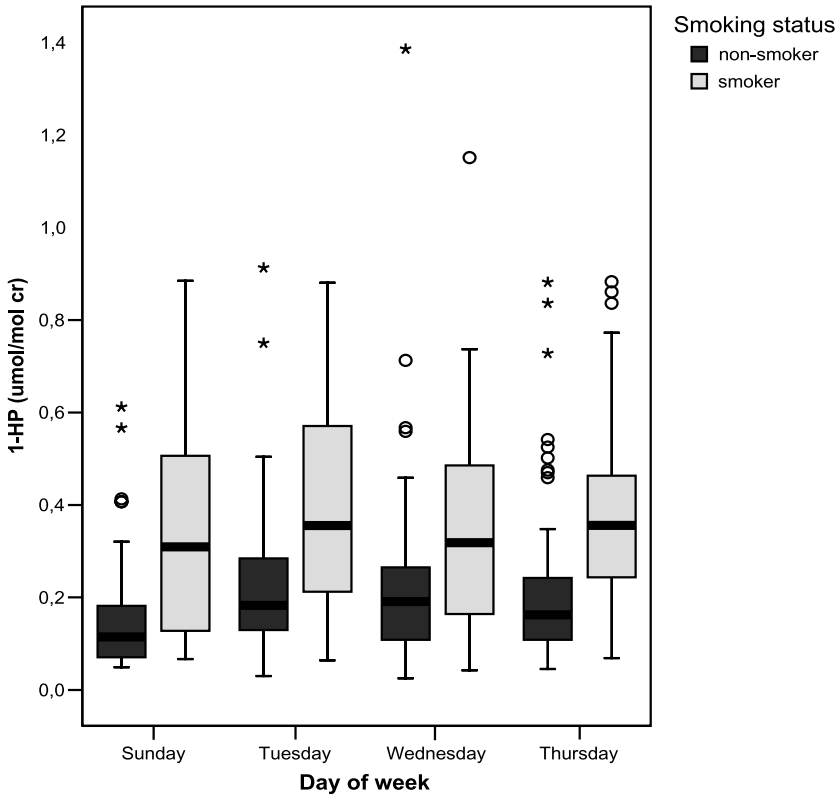

Figure 1. Urinary 1-hydroxypyrene levels in rubber manufacturing workers by day of urine collection. Each box shows the median, 25th and 75th percentiles. ○, outliers (values between 1.5 and 3 times the interquartile range). *, extreme values (>3 times the interquartile range). *1-HP,* 1-hydroxypyrene; *cr,* creatinine.

in urinary 1-hydroxypyrene was not uniform across tasks and factories; largest increases were observed in the molding, curing, and finishing department, wherein it was significantly related to inhalation and dermal exposure to cyclohexane soluble matter. Urinary mutagenicity was significantly increased during the workweek for compounding, mixing, and curing, which seemed to be associated with inhalable dust exposure. PBMC DNA adducts showed no clear association with production functions or with internal and external exposure measures. However, clear associations were found between urothelial cell DNA adducts and working in the compounding, mixing, and curing departments. Total urothelial cell DNA adducts levels were significantly related to urinary 1-hydroxypyrene and mutagenicity. For 1-hydroxypyrene, this correlation was mainly driven by adduct 3, whereas adduct 1 was more strongly associated with mutagenicity. No significant relation was found between the identified PBMC and urothelial cell DNA adducts.

1-Hydroxypyrene levels were as much as twice as high during the workweek compared with Sunday. These results are in concordance with a previous study among Swedish rubber workers that showed on average 200% higher 1-hydroxypyrene levels in exposed workers than in controls (23). Although in our study, it is possible that we underestimated the contribution of occupational exposures to 1-hydroxypyrene levels because of the accumulation of PAHs in the adipose compartment. 1-Hydroxypyrene levels in occupationally exposed subjects might not be reduced entirely to background levels over the weekend. However, Sunday urinary 1-hydroxypyrene levels were not related to department or factory. By using the individual Sunday urine sample as control (collected ~48 h after last exposure), interindividual variation will not influence the results, which, given these low exposures, is essential to be able to detect small differences in urinary 1-hydroxypyrene levels. The same is true for the mutagenicity analyses, wherein Sunday levels may also still be elevated because of exposures during the previous workweek (19). The strong correlation (r = 0.79-0.99; $P < 0.05$) between Sunday and pooled weekday mutagenicity levels for the production functions with the highest exposures seemed to confirm this hypothesis. However, because the mutagenic compounds are still unknown, it is impossible to judge the likelihood of the delayed excretion of these compounds, leading to an elevated urinary mutagenicity on Sunday.

Compared with other industries like aluminum production or coke ovens, PAH exposure among rubber workers is rather low. Mean weekday urinary 1-hydroxypyrene level for all rubber manufacturing workers in our study population was 0.22 μmol/mol creatinine, with the highest levels among curing workers (0.31 μmol/mol creatinine). This is still within the range of what is normally regarded as representative levels in non–occupationally exposed individuals in Western Europe and Canada (0.03-0.8 μmol/mol creatinine; ref. 24), and is below the current American Conference of Governmental Industrial Hygienists biological exposure index of 0.5 μmol/mol creatinine(25), which indicates significant occupational exposure. Nonetheless, exposures at these levels seemed to be correlated with increases in target organ DNA adduct levels.

Table 2. Urinary exposure biomarker levels by department

	K*	Sunday urine			Pooled weekday urine			Individual effect (median), %	P †
		AM	GM	GSD	AM	GM	GSD		
Urinary 1-hydroxypyrene (μmol/mol creatinine; K = 115)									
Compounding and mixing	10	0.37	0.30	2.31	0.30	0.23	2.23	+23	0.4903
Pretreating	16	0.25	0.17	2.52	0.27	0.21	2.05	+42	0.3058
Molding	28	0.20	0.14	2.24	0.25	0.21	1.81	+54	0.0620
Curing	28	0.25	0.19	2.13	0.38	0.31	1.99	+99	0.0034
Finishing	11	0.28	0.22	2.15	0.32	0.26	1.90	+52	0.5534
Shipping	4	0.15	0.12	2.15	0.18	0.15	1.81	+32	0.1138
Engineering	16	0.15	0.13	1.88	0.21	0.17	1.95	+45	0.1232
Laboratory	2	0.11	0.11	—	0.07	0.06	1.86	−63	—
Urinary mutagenicity (revertants/g creatinine; adjusted for cotinine; K = 104)									
Compounding and mixing ‡	8	6,522	4,743	2.44	10,511	7,051	2.74	+56	0.0204
Pretreating	14	3,309	1,612	4.96	5,631	4,190	2.21	+22	0.0792
Molding ‡	25	12,986	6,515	3.57	10,645	5,194	4.58	−15	0.2763
Curing ‡	27	10,447	5,553	3.33	11,527	8,240	2.21	+21	0.0499
Finishing ‡	11	15,672	7,555	3.66	12,465	7,134	3.06	−20	0.8059
Shipping ‡	3	9,678	7,928	2.15	11,163	9,169	2.11	+17	0.1185
Engineering	14	6,925	2,550	6.09	9,355	6,746	2.23	+68	0.0793
Laboratory	2	4,453	3,968	2.00	1,969	802	8.91	−67	0.3719

Abbreviations: AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation.
*K = total number of subjects.
†Mean weekday urine tested against individual Sunday urine sample (paired t test).
‡$P < 0.05$ for Pearson's correlation between Sunday and pooled weekday mutagenicity level (r = 0.79-0.99).

Workweek 1-hydroxypyrene levels are essentially the product of the current day exposure, whereas DNA adduct levels integrate daily exposure over ~100 days, the lifespan of the urothelium (26). Therefore, relatively small increases in daily exposure can have a larger cumulative effect on the adduct level. This has been reported earlier for persons with environmental tobacco smoke exposure (27).

In the rubber manufacturing industry, the highest exposure levels are likely to occur in the compounding and mixing departments, wherein most handling, weighing, and mixing of raw materials, including extender oils and carbon black, takes place. Highest process temperatures are reached in the curing department, which likely contributes to that exposure. Compounding, mixing, and curing workers had also increased urinary mutagenicity levels in addition to elevated 1-hydroxypyrene levels. However, there was no significant correlation between these markers on an individual basis. Because the YG1041 strain is not PAH specific, this is not totally unexpected. These data, together with the dissimilar associations to external exposures, indicate that genotoxic risks in the rubber manufacturing industry might be mainly driven by exposures other than PAHs. Nevertheless, because a possible association was observed between 1-hydroxypyrene and urothelial cell DNA adducts, particularly adduct 3, the data confirm our initial observation that PAHs might play some role in urinary bladder carcinogenesis in the rubber manufacturing industry (8). However, PAHs are not specific bladder carcinogens, and the fact that we did not find an association between 1-hydroxypyrene and PBMC adduct levels is cautionary against making an association. It might be more likely that concomitant exposures through perhaps disparate routes are the joint cause of the elevated urinary adduct levels. The correlation we found between bladder adducts and urinary mutagenicity has also been observed in the general population (28, 29). Vineis et al. (29) suggested that, in the general population, DNA damage in the urothelium seems to be mainly due to arylamines (largely through tobacco smoke).

Because the exposures of rubber workers are so complex, it is difficult to determine the causal agent(s). However, we found some interaction of acetylation status on the correlation between urinary mutagenicity

Table 3. Association between personal inhalable dust, rubber fumes measured as cyclohexane soluble matter, dermal cyclohexane soluble matter exposure, and urinary levels of 1-hydroxypyrene and mutagenicity

	Exposure*	β (SE)	P
Urinary 1-hydroxypyrene † (K = 81; n = 222) ‡	Inhalable dust	−0.05 (0.08)	0.5060
	Rubber fumes (cyclohexane soluble matter)	0.29 (0.13)	0.0244
	Dermal cyclohexane soluble matter	0.13 (0.05)	0.0163
Urinary mutagenicity † (K = 103; n = 103) ‡	Inhalable dust	0.22 (0.13)	0.0786
	Rubber fumes (cyclohexane soluble matter)	0.15 (0.20)	0.4341
	Dermal cyclohexane soluble matter	0.03 (0.09)	0.7321

*Exposures were entered in one model as continuous variables after natural log transformation.
†Adjusted for urinary cotinine and Sunday biomarker level.
‡K = total number of subjects; n = total number of samples included.

and bladder DNA adducts, indicating a higher genotoxic risk for slow acetylators. This would be consistent with arylamine exposure being associated with aromatic amines (30), as a recent meta-analysis pointed to a role of NAT2 acetylator genotype in urinary bladder cancer. Results from earlier studies on mutagenic exposures among rubber manufacturing workers have pointed toward the importance of aromatic amine exposure for genotoxic risk in the rubber manufacturing industry as well (7, 31).

We found no correlation between bladder and blood cell DNA adduct levels, suggesting that the adducts in each case are potentially formed by different agents and/or by different metabolic actions. Neither did we find any association between the PBMC adducts and any occupational exposure measures (production functions, external and internal exposure levels). However, in urothelial cells, we observed that the highest exposed workers, which are those in the compounding, mixing, and curing departments, had significantly higher adduct levels compared with the other workers. The fact that we did not observe any correlation between adduct levels in urothelial cells and PBMCs indicates the presence of specific carcinogens that only interact with DNA in the bladder lumen. This is again consistent with aromatic amine exposure, wherein the carcinogens circulate in the blood as glucuronides, which are then cleaved by acid hydrolysis in the urinary bladder (32). Although we cannot pinpoint a specific agent to the observed bladder adducts, the finding is of importance because subjects with elevated bladder DNA adduct levels have been found to have an increased risk (odds ratio, 1.9; 95% confidence interval, 0.8-4.3) for developing bladder cancer (33).

The results of this study indicate that, at present, compounding, mixing, and curing workers are at highest genotoxic risk among rubber manufacturing workers, which is in keeping with epidemiologic findings on cancer risks in this industry (1). Urothelial cell DNA adducts were not related to PBMC DNA adducts, hinting possibly to the presence of specific bladder carcinogens in the rubber manufacturing industry. However, PAH exposure is not considered a major cause of the found genotoxic risk.

Table 4. Prevalence (%) and mean RAL ($\times 10^7$) of urothelial cell and PBMC DNA adducts of positive samples

	n* (%)	Mean (SD) †
Urothelial cell DNA adducts (n = 52)		
Adduct 1	30 (58)	3.65 (3.15)
Adduct 2	12 (23)	6.24 (5.11)
Adduct 3	23 (44)	4.54 (3.33)
Adduct 11	2 (4)	12.6 (5.30)
Total	38 (73)	8.26 (8.01)
PBMC DNA adducts (n = 48)		
Adduct A	5 (10)	8.68 (5.70)
Adduct B	38 (79)	6.43 (6.69)
Adduct C	3 (6)	10.5 (5.56)
Adduct D	23 (48)	3.41 (2.30)
Total	40 (83)	9.72 (10.8)

*Number of subjects with adduct levels of $\geq 1 \times 10^7$ RAL.
†Mean of positive samples.

Table 5. Association between 1-hydroxypyrene, urinary mutagenicity, urothelial, and peripheral blood cell DNA adducts among rubber manufacturing workers

	n	β (SE)	P
Model with urinary 1-hydroxypyrene* as predictor			
Urinary mutagenicity*,†	103	0.18 (0.18)	0.3151
Urothelial cell adducts (total) ‡	50	1.4 (0.68)	0.0349
Adduct 1 ‡	50	0.41 (0.48)	0.3874
Adduct 3 ‡	50	0.92 (0.49)	0.0579
PBMC adducts (total) ‡	45	−0.80 (0.65)	0.2165
Model with urinary mutagenicity* as predictor			
Urothelial cell adducts (total)‡	51	0.75 (0.34)	0.0271
Adduct 1 ‡	51	0.59 (0.29)	0.0398
Adduct 3 ‡	51	0.35 (0.26)	0.1746
PBMC adducts (total)	46	−0.58 (0.46)	0.2031

*Weekday biomarker level.
†Linear regression.
‡Logistic regression; adjusted for cotinine and NAT2.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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