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2-Naphthol levels and genotoxicity in rubber workers

Glenn Talaska[a,*], Beverly Gaultney[a,1], Susan Peters[b], Paul Succop[a], Roel Vermeulen[b]

[a] Department of Environmental Health, University of Cincinnati College of Medicine, 3223 Eden Ave, Cincinnati, OH 45267-0056, United States
[b] Environmental Epidemiology Division, Institute for Risk Assessment Sciences, Utrecht University, The Netherlands

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ABSTRACT

Urinary bladder cancer is a historical disease of rubber workers often been associated with exposure to aromatic amines such as 2-naphthylamine. While exposure to these compounds has decreased markedly over time, the bladder cancer risk has not decreased in direct proportion. Polycyclic aromatic compounds (PAC) are candidates for urinary bladder cancer causation. We determined pre- and post-exposure urinary levels of 2-napthol (2NAP), the major metabolite of a model volatile PAC, in a group of non-smoking rubber workers. Pre- and post-exposure urine samples were collected from 43 non-smoking workers. Overall mean post-shift 2-naphthol levels were increased (13.95 ± 28.4 μg/l), but non-significantly compared to samples collected pre-exposure (7.97 ± 22.1 μg/l; $p = 0.29$). The greatest difference was observed in the curing department where post-exposure samples were 4.5 fold higher, post shift samples were significantly higher in production workers as compared to non-production workers ($p = 0.02$). Levels of 2NAP were not correlated with levels of carcinogen-DNA adducts in exfoliated urothelial cells nor with other estimates of exposure or effect. These data suggest that post-shift urinary 2NAP levels are increased, particularly in the curing department. However, the differences were not significantly different overall and urinary 2NAP levels did not predict the level of carcinogen DNA adducts in exfoliated urothelial cells.

1. Introduction

Aromatic amines such as 2-naphthylamine have long been associated with increased rates of urinary bladder cancer (UBC) in rubber workers (Talaska, 2005). Several research groups have reported that an increase in UBC persisted – at about twice expected – even though the putative exposures to aromatic amines decreased over time (Kellen et al., 2007; Reulen et al., 2008; Straif et al., 1998; Straughan and Sorahan, 2000). However, Dost et al. (2007) recently saw the risk diminish to about 1.25 times expected in a cohort of workers hired after 1980, a level that was not statistically significant. So while it appears that aromatic amines were in fact the largest contributors to UBC in rubber workers, the possibility exists that other, uncontrolled compounds may still be contributing to an increased risk of the disease.

Exposure to polycyclic aromatic compounds (PAC) has been associated with UBC in several industries (Doll et al., 1972). A review of studies involving industries and environments showed increases in UBC as a common finding where there is PAC exposure, although in individual studies statistical significance is often not reached due to small numbers of participants and cases (Boffetta et al., 1997). Nonetheless, industries with exposures to coal tars and pitches were associated with significant increases in UBC. These include aluminum reduction workers often with a clear dose–response relation with years of employment, coal carbonization and gasification workers, some increases in carbon black workers and roofers. Romundstad et al. (2000) reported an increase in UBC in workers employed at two Norwegian aluminum smelters, the increased was confined to those employed for more than 3 years. They also showed a dose response in UBC with increased estimated PAC exposure. Moulin et al. (2000) also reported that French aluminum reduction workers were at 1.77 fold higher risk for UBC, but the confidence intervals in this case included one. Using biomarkers Simioli et al. (2004) reported that non-smoking coke oven workers excreted increased levels of urinary mutagens. Similar results have been reported in several other studies while these studies do not conclusively show that PAC contribute to UBC, they certainly suggest that the possibility of an association exists.

PAC are common potential exposures in rubber workers. Extender and process oils are used to swell the rubber fibers, to speed processing to ease release of the rubber materials for equipment such as molds. Aromatic oils which may contain as much as 30% of 4–6 ring PAC are common because they posses technical characteristics

Abbreviations: UBC, urinary bladder cancer; PAC, polycyclic aromatic compound(s); 1HP, 1-hydroxypyrene; BAP, benzo(a)pyrene; 2NAP, 2-naphthol; CSM, cyclohexane soluble matter.

* Corresponding author. Tel.: +1 513 558 0519; fax: +1 513 558 4397.
E-mail address: glenn.talaska@uc.edu (G. Talaska).
[1] Current address: PPG Industries.

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that are desirable for process and final product (Maarufa Ltd, 2009). Tires may also contain as much as 50% carbon black by weight. Carbon blacks greatly enhance the durability and abrasion resistance of the finished product. PAC are often associated with carbon black, but since the affinity of the PAC for the carbon structure is high, it is thought that very little is released. This has been shown experimentally when aqueous media is used as the extracting material. However, substantial PAC may be released by soxhlet extraction. In addition, Gerde et al. (2001) saw that benzo(a)pyrene (BAP) coated on to soot particles was very bioavailable in the alveolar regions of the lung of dogs and was subsequently metabolized. They showed that 85% of the BAP was bioavailable in 15 minutes. These data would suggest that at least some absorption may be taking place *in vivo*.

The focus of this research program is to determine the role that PAC may play in UBC in rubber workers. We have investigated the relationship between PAC external dose and the levels of carcinogen–DNA adducts in exfoliated urothelial cells as an indicator of the level of genetic damage to the target organ (Vermeulen et al., 2002). We reported workers employed in the mixing and curing areas had the highest DNA adduct levels. Fast acetylators had higher adduct levels in general. We also reported that the levels of urinary 1-hydroxypyrene (1HP), a biomarker of PAC internal dose, were elevated following exposure and that total adduct levels and levels of one specific DNA adduct were correlated significantly with 1HP levels. Urinary mutagenicity in strain YG1041, a strain designed to respond to aromatic amines, also predicted total adduct levels, but was specifically related to a different adduct than which was predicted by 1HP. These findings, along with the acetylation results mentioned above would not be expected if aromatic amines were the only source of the adducts seen (Bartsch et al., 1990). Workers in the curing and finishing departments had the highest levels of 1HP and DNA adducts and these levels were associated suggesting that exposure to compounds that behave like pyrene were causing the formation of DNA adducts in the exfoliated cells. Pyrene is 4 ring non-carcinogenic PAC with physical properties similar to, but not identical with other 4–6 ring carcinogenic PAC (ACGIH, 2009). The focus of the present work was to determine if exposure to vapor phase PAH was related any particular carcinogen DNA adduct or to total DNA adducts. A positive finding would suggest that exposure to vapor phase PAH would carry a significant risk of genetic insult and would help to direct intervention strategies against these exposures. Naphthalene is a PAC with 2 aromatic rings and has significantly higher vapor pressure and lower boiling point than the larger compounds. It is generally more prevalent in PAC mixtures than is pyrene and/or BAP. In this study we wanted to determine if exposure to more volatile PAC would be related to DNA adduct levels in exfoliated urothelial cells. Naphthalene, like pyrene is not considered to be carcinogenic, but like 1HP, 2NAP serves as an easily measured surrogate for compounds in a PAH mixture which have similar physical properties and are likely absorbed by the same mechanisms.

2. Materials and methods

Urine samples from 43 nonsmoking rubber workers were obtained and analyzed for all the markers reported. This is a subset of the 56 workers who participated in earlier studies as sufficient urine to perform all analyses was not obtained in every case. The external exposure and other measurements were described earlier (Peters et al., 2008; Vermeulen et al., 2002). In brief, spot urine samples were collected before exposure on Sunday and then after the work shift 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 (for isolation and analysis of exfoliated urothelial cells) were collected from Monday (after first morning void) to Tuesday (including first morning void). These samples were kept cold (0 °C) during collection and 10% glycerol (volume for volume) was added after collection to minimize cell loss due to lysis after freezing (−20 °C).

2-Naphthol (2NAP) is a major metabolite of naphthalene. Due to its lower molecular weight a larger fraction of naphthols are excreted in the urine than for metabolites of other PAC. 2NAP was selected for analysis since it appears to be more influenced by occupational exposure than tobacco smoking. Analysis of urine samples for 2NAP was conducted in a fashion similar to described earlier for 1HP (Peters et al., 2008; Vermeulen et al., 2002). Samples were solid phase extracted, eluted, dried and resolublized in methanol before being injected into a Waters HPLC system which included a 2960 solvent delivery system, a Waters Novapak C18 column (4 μm particle size, 2 by 150 mm), and a Waters 474 fluorescence detector set to 227 nm excitation and 355 nm emission. The system was controlled and the raw data analyzed with Waters Empower software. Methods used for urinary mutagenicity and DNA adduct analysis by ^{32}P-postlabeling in both white blood and exfoliated urothelial cells and analysis of 1-hydroxypyrene levels were described earlier (Peters et al., 2008; Vermeulen et al., 2002).

The study was approved by the institutional review board. Participation was voluntary, and written informed consent was obtained from participants.

3. Results

Table 1 presents the urinary 2NAP levels for the different departments, pre- and post shift. Overall mean post-shift 2NAP levels were increased (13.95 ± 28.4 μg/l), but non-significantly compared to samples collected pre-exposure (7.97 ± 22.1 μg/l; $p = 0.29$). Average values were greater in the post-exposure samples, but the difference was not statistically significant ($p = 0.29$). There were no significant differences in 2NAP levels in pre- and post-exposure urine samples by department. However, the greatest fold difference was in the curing department ($p = 0.06$). Production workers were employed in the compounding and mixing, pre-treating, molding, or curing department had significantly higher post shift 2NAP levels than did non-production workers ($p = 0.02$).

Table 2 presents the effect of factory and department on the levels of biomarkers including those reported earlier (Peters et al., 2008). The probability values for tests of differences with various models are provided in the table. Urinary 2NAP levels are driven by the individual factory more than any other of the biomarkers. As reported earlier, department was the major driver of levels of other biomarkers, 1HP and DNA adducts in exfoliated urothelial cells. When the factory × department interaction was introduced into the model (Model 4), the main effect of factory was no longer significant on urinary 2NAP level, suggesting that some factors within specific factories other than department were the major reasons for the 2NAP levels seen. On the other hand, while the main effect of department remained significantly related with 1-HP and

Table 1
Pre- and post-shift 2NAP[a] levels in rubber workers by department.

Production	*N*	Pre-shift			Post-shift		
		AM	GM	GSD	AM	GM	GSD
Compounding and mixing	4	13.3	2.24	16.5	22.5	7.65	12.1
Pre-treating	8	6.5	0.74	7.9	17.6	1.38	11.9
Molding	13	13.0	0.67	9.4	18.4	1.35	13.6
Curing	13	1.9	0.4	4.4	9.0	0.66	8.8
Non-production	5	8.9	1.3	13	2.5	0.5	5.8
Overall	43	7.97	0.71	7.8	13.95	1.15	10.5

[a] Levels are expressed as micrograms per liter of urine. *Abbreviations used:* arithmetic mean (AM), geometric mean (GM), geometric standard deviation (GSD), and the number of persons in the group (N).

Table 2
p-values for type 3 tests of fixed effects of factory and department on exposure and early effect markers.

Model[a]	Fixed effect	*p*-values				
		1-HP[b,c]	Mutagenicity[b,d]	Bladder adducts[d]	PBMC adducts[d]	2-Naphtol[b,d]
1	Factory	0.1743	0.6874	0.6908	0.6047	**0.0217**
2	Department	**0.0191**	0.0582	**0.0001**	0.9136	0.6559
3	Factory	0.1798	0.8036	0.8879	0.4748	**0.0212**
	Department	**0.0250**	0.1057	**0.0016**	0.7280	0.3902
4	Factory	0.1059	0.8005	0.0890	0.8512	0.1361
	Department	**0.0104**	0.3360	**<0.0001**	0.7704	0.7920
	Factory × Department	**0.0038**	0.9302	**0.0029**	0.9141	0.6490

Significant (0.05) effects are bolded.
[a] 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 effect; (4) Multivariate model with factory and department as fixed effects and the interaction between factory and department.
[b] Urinary 1-HP, mutagenicity and 2-naphtol are corrected for individual Sunday biomarker and cotinine level.
[c] Mixed effect models.
[d] General linear models.

bladder adducts, suggesting that these outcomes were influenced by departments regardless of specific factory. The interaction was also significant for these 2 outcomes, indicating that the effect of department was stronger in certain factories than in others.

There was no association between urinary 2NAP levels and any of the measurements of external exposure made, namely, inhalable dust, cyclohexane soluble matter (CSM) in air filter samples or CSM on dermal skin wipes. 2NAP was not correlated to 1-HP in post-exposure urine samples. In addition there was no correlation between 2NAP levels and levels of carcinogen DNA adducts in either blood leukocytes or exfoliated urothelial cells (data not shown).

4. Discussion

The rubber manufacturing process is extremely complicated and involves exposure to a large variety of agents in all three physical states and by dermal contact and inhalation. Workers in different stages of the process may be exposed to dusts, vapors and a combination. We previously reported that levels of 1HP and DNA adducts in exfoliated urothelial cells were increased in rubber workers, particularly in the curing departments of the factories we studied. 1HP levels were a significant predictor of DNA adduct levels (Peters et al., 2008) and increases in the DNA adduct levels were seen at levels of 1HP below the current ACGIH BEI benchmark (ACGIH, 2009). Levels of 1HP in urine have been previously shown to be a good predictor of exposure to carcinogenic PAC (ACGIH, 2009). One adduct in particular was related to 1HP levels, suggesting either that it may have originated in a PAC exposure or that the exposure route and pathway of the causal agent was similar to that of pyrene. Since urinary 1HP was earlier shown to be related to cyclohexane soluble extracts in particulate collected from the workplace, we undertook a study to determine if levels of 2NAP provided an additional dimension to the exposure and effect scenario we were discovering. Naphthalene differs from many carcinogenic PAC in having a much lower boiling point and higher vapor pressure. Indeed in the present study urinary 1HP levels were not correlated to 2NAP in post-exposure samples, therefore it appears that exposure to these compounds may be by different mechanisms and that 2-NAP do provide another exposure dimension compared to 1-HP. We report here that 2NAP, the major exposure-related metabolite of naphthalene exposure, is increased, but not significantly so, in post-shift samples of non-smoking rubber workers due mostly to the wide variation in values seen in the population. Workers in the curing department had the greatest fold increase in their post-exposure samples although this increase was not formally statistically significant ($p = 0.06$). Production workers and non-production workers had similar pre-exposure levels but post-exposure levels of 2NAP were higher in production workers ($p = 0.02$), suggesting that 2NAP levels do capture an exposure different from 1HP. 2NAP levels were not related to markers of external exposure as measured by inhalable dust or CSM in the dust. Unfortunately no samples of the vapor phase were collected. We cannot explain the high level of 2NAP in the pre-exposure (Sunday) sample, other than to note that very high values were seen in 2 individuals.

In our earlier work we saw that levels of DNA adducts in peripheral blood leucocytes was not correlated with levels of 1HP, urinary mutagenicity or with DNA adducts in the exfoliated urothelial cells. We note here that the leucocyte adduct levels are also not correlated with 2NAP levels. In sum, these data call into question the utility of measurement of DNA adducts in leucocytes in PAH exposure situations (Wiencke, 2002; Zhou et al., 1997).

In conclusion, these data suggest that post-shift urinary 2NAP levels are increased among rubber production workers, suggesting exposure to vapor phase PAH in certain factories. However, the differences were not significantly different overall and urinary 2NAP levels did not predict the level of carcinogen DNA adducts in exfoliated urothelial cells or the excretion of urinay mutagens. Thus, it appears that vapor phase PAH do not contribute to the burden of genetic damage seen in rubber workers and that interventions should be directed at reduction of exposure to PAH on particulate and via the dermal route.

Conflict of interest

The authors declare that there are no conflicts of interest.

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