Title: Target Organ DNA Damage in Coke Oven Workers

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List of Abbreviations: 1HP: 1-hydroxypyrene; ACGIH: American Conference of Governmental Industrial Hygienists; BEI: Biological Exposure Index; HPLC: high pressure liquid chromatography; PAH: polycyclic aromatic compounds; TLV: Threshold Limit Value;

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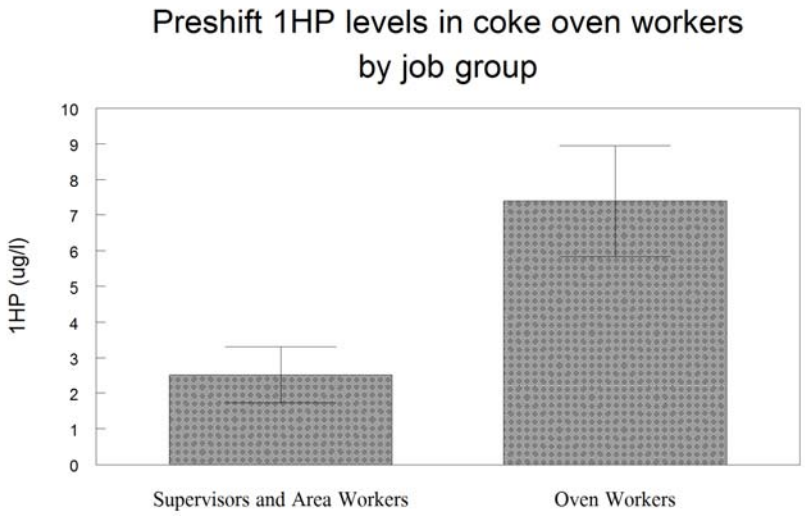

Figure 1: Levels of 1HP by job group. Groups were defined by whether they worked on or off the coke ovens. Supervisors (n=3) and side area workers (n=9) were pooled after it was determined that there was no difference in 1HP levels. Similarly the top oven and side oven workers were pooled in the analysis as there was no difference between their values. The mean levels are 2.51 ug/l (SE=0.79) (supervisors and area workers) and 7.4 ug/l (SE=1.56) (oven workers); there was a statistically significant difference ($p<0.02$) between these levels.

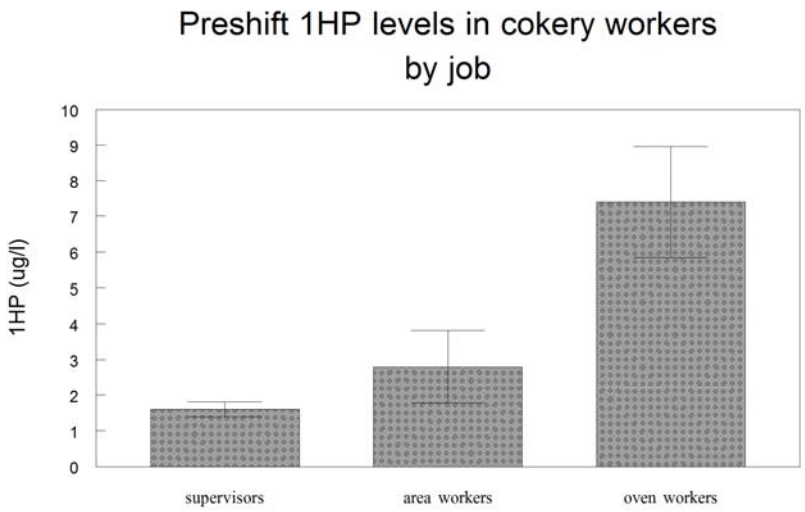

Figure 2: Levels of 1HP by working group showing the separate levels in the non-oven workers. There were statistically significant differences between both the supervisors (mean= 1.6 ug/l, se=0.21, $p<0.05$), the area workers (mean= 2.79, se=1.02, $p<0.05$) and the oven workers (see above).

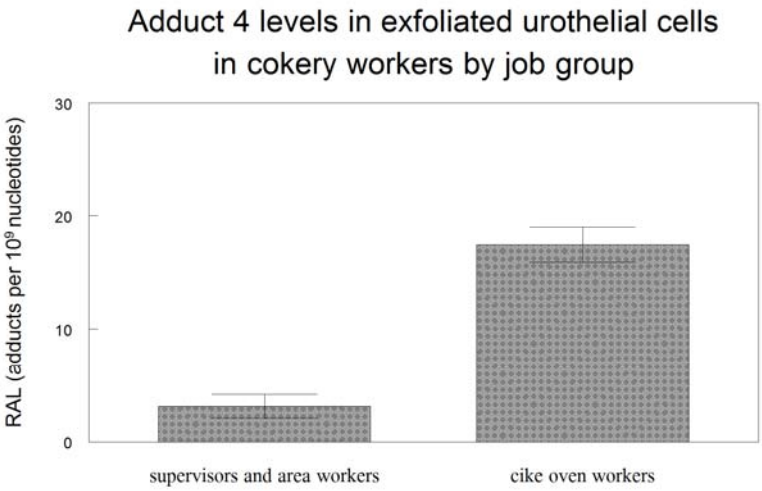

Figure 3: Levels of DNA Adduct 4 in cokery workers by job grouping. Job groups are as in figure 1. The mean level of adduct 4 in supervisors and area workers was 3.12 adduct per 10^9 unadducted nucleotides (se= 1.05) and that in the oven workers was 17.43 adducts per 10^9 unadducted nucleotides (se=4.61). This difference was statistically significant at $p<0.02$.

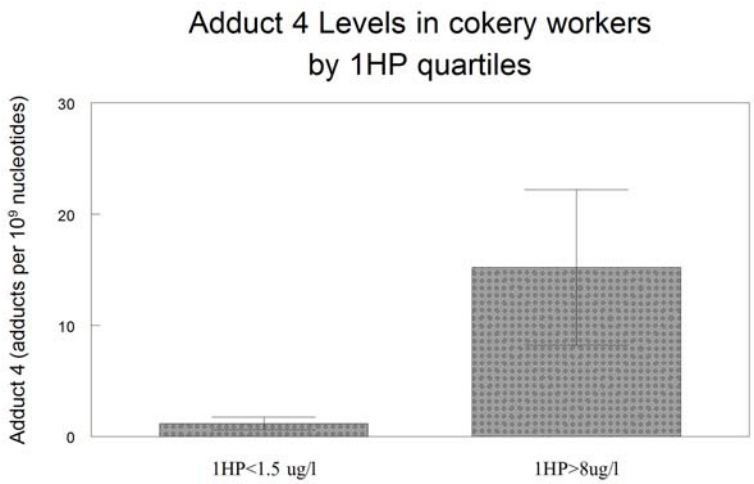

Figure 4: Levels of DNA adduct 4 in cokery workers by lowest versus upper quartile of 1HP values. Workers were arrayed by their 1HP levels and the upper and lower 25% compared. The mean values in the lowest quartile was 1.56 adducts per 10^9nucleotides (se=0.53) and in the highest was 29.97adduct per 10^9nucleotides (se=5.46). This difference was statistically significant at $p<0.01$.

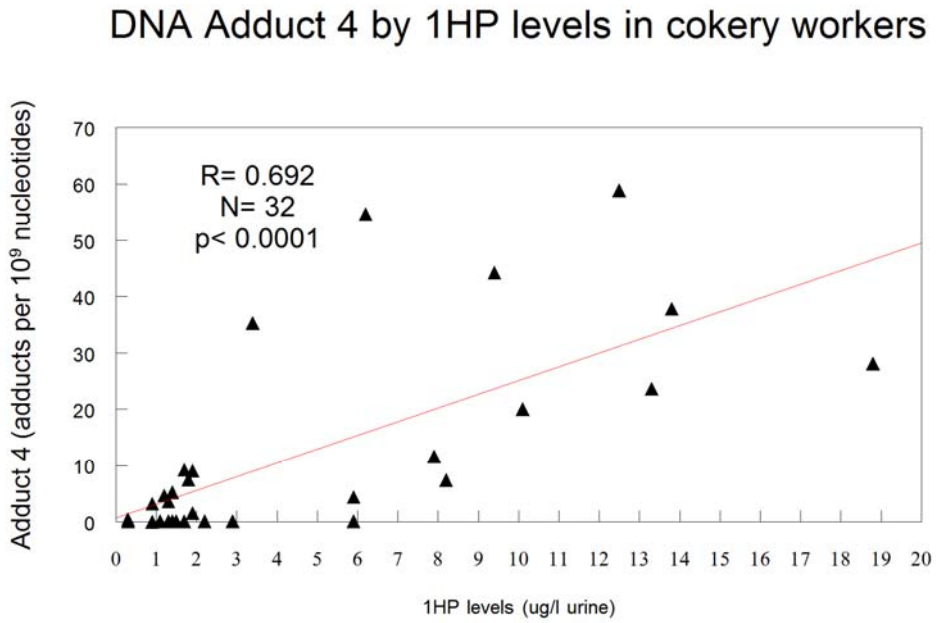

Figure 5: Levels of DNA adduct 4 in exfoliated urothelial cells by levels of urinary 1 HP. The equation for the line is y=2.44x+0.74.

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There are no tables provided.

Abstract

Making accurate exposure measurements for polycyclic aromatic hydrocarbons (PAH) is a difficult problems facing the occupational health professional. PAH have low acute toxicity, but many are recognized carcinogens. They have a low vapor pressure, but can be adsorbed onto inhaled particulates and are also readily absorbed through the skin. When the materials are heated, they produce dusts, vapors or fumes; and while respirators are used to limit uptake, it is difficult to estimate their efficiency in practice. The impact of differences in body fat and activity (respiration rate) are simply unknown. For these reasons, biological monitoring is being used increasingly to estimate exposure. The credo of industrial hygiene is "to protect the health of the worker." One of the major advances to this end has been the development of exposure values for professional practice, e.g., the TLVs and BEIs. A BEI has been established for PAH exposure using the internal dose marker 1hydroxypyrene (1HP) as the index. However, due to data limitations, this BEI is not related to health effects directly or indirectly (as a TLV – equivalent). Instead, the BEI was set as an advisory level using the distribution levels in the general population including the levels seen in tobacco smokers. The epidemiology of tobacco smokers clearly indicates excess lung and urinary bladder cancer risks in this population at 1HP levels that are lower than those seen in many occupational groups. While it is acknowledged that tobacco smoke contains many materials other than PAH which may add to its carcinogenicity, basically, this means that it is not known whether the BEI protects workers from the most significant health effects of PAH exposure, genotoxicity and cancer.

The specific aims of this study were to:

1. Determine the relationship between urinary levels of 1-hydroxypyrene (1HP) and levels of carcinogen-DNA adducts in exfoliated urothelial cells (urine sample sediments). Determine if a dose response exists and the degree of association. These data will allow us to determine at what point 1HP levels are related to significant increases in PAH DNA adduct levels in the target organ, the urinary bladder. If such a level exists, it will be important in setting health exposure guidelines for PAH that are based on human health effects instead of background population levels, as they now are. There is some indication that the current BEI for PAH may not prevent the occurrence of genetic damage. This study will help to determine if that is the case and supply important data needed to revise the BEI if so indicated. These internal dose levels will be compared to air PAH levels, data which has been collected by our collaborators in Germany.

2. Determine the levels of 1HP and 1HP glucuronide using HPLC techniques and our recently developed sensor probe which could potentially be used to measure each of these biomarkers in real time.

This work contributes directly to the NORA2 goals of reducing cancer in Manufacturing by contributing to the information base on the extent of PAH carcinogen exposure and

Significant Findings

We report that many coke oven workers have exposure that greatly exceed the end of shift BEI for 1HP of 1 ug/l in their pre-shift samples. This was particularly true in the workers who worked on the coke ovens themselves (G2). Oven workers (G2) had average 1HP levels of 7.4 ug/l as opposed to side area workers and supervisors (G1) whose levels averaged 2.5 ug/l ($p<0.02$). We investigated what impact there levels would have on the levels of DNA adducts in the exfoliated urothelial cells of the workers. We saw that the levels of one adduct (Adduct 4) was significantly correlated with the levels of 1HP in workers ($r= 0.691$, $p<0.001$). This suggests that Adduct 4 in the urothelial cells is related to PAH exposure either by being caused by a PAH, or by being caused from exposure to another compound which has exposure characteristic very similar to PAH in this workplace. The correlation was slightly stronger in the workers who did not work in the ovens ($r=0.75$) than in those who did, suggesting that the high exposures in the oven workers may also be quite variable. In addition, we saw that workers in the lowest quartile of 1HP values (average 1.0ug/l) had an average Adduct 4 level of 1.3 adducts per 10^9 nucleotides, while the workers in the highest quartile of 1HP values (>8 ug/l) had an adduct level that was 15.2 times higher ($p<0.001$). No other adducts were related to 1HP levels or were significantly different between the 2 groups. In addition, there was no significant impact of tobacco smoking on adduct levels in any group. These data suggest that exposure in these operations remains almost completely uncontrolled and should be a point of emphasis for regulatory agencies. These data suggest coke oven workers remain at significantly increased risk of genetic disease due to their exposures to PAH. In addition, adduct levels begin to increase approximately at a 1 HP level of 1.0 ug/l.

Usefulness of Findings

These data are directly applicable to the development of a health-based BEI for PAH metabolites. In addition, the development of a sensor for PAH metabolites will be extremely useful in making real-time measurements. These measurements will allow us to better understand the kinetics of absorption and elimination of PAH in humans. Once this is accomplished we will be in an excellent position to provide feedback to workers about which specific tasks and actions lead to increased elimination of PAH metabolites. This should lead to reduced exposures in many workplaces.

Scientific Report

Regarding specific aim one we collected pre-shift urine samples 32 coke oven workers at various job locations. 1-HP and DNA adduct levels were analyzed according to methods as published previously. In brief, the samples were filtered to remove cellular components which were reserved for DNA adduct analysis. The supernatant was then hydrolyzed to deconjugate metabolites. Solid phase extraction was used to concentrate metabolites. 1-HP levels were determined by high performance liquid chromatography (HPLC) using a fluorescence detector at an excitation wavelength of 242 nm and an emission wavelength of 388 nm. Standard curves were used to quantitate the levels. DNA was extracted from the isolated urothelial cells and analyzed for DNA adducts using ^{32}P-postlabeling according to our published procedures (Talaska, Schamer et al. 1991, Rothman, Bhatnagar et al. 1996) .

Pre-shift levels of 1 HP were used in this study because they are more likely to reflect the chronic exposure of the workers better than the end of shift samples which are more likely to be a function of the day of sampling exposure(ACGIH 2011).

The American Conference of Governmental Industrial Hygienists (ACGIH) suggests that a level of 1-HP in urine greater than 1 µg/l should be considered indicative of occupational exposure to PAHs. However, the relationship between 1HP levels and health effects has yet to be determined. A focus of this project was to determine, if possible, whether the ACGIH BEI was protective of worker health by determining at what 1HP level were increases in DNA adduct levels in the target organ observed.

We report that 28 of the 32 workers had pre-shift 1HP levels greater than the ACGIH recommendation, which is based on post-shift values. This indicates that exposures are poorly controlled in this industry. Analysis of job category indicated that top oven workers and top oven workers with top oven/side oven workers were significantly more exposed compared to supervisors and side area workers as the control group. Figure 1 shows that there was a statistically significant increase in 1HP levels in workers who worked directly on the ovens (top and side oven workers) compared to those workers who did not work directly on the ovens (supervisors and side area workers). The 1HP levels we report are consistent with those reported for coke oven workers Germany, Poland and China(Mielzynska, Braszcynska et al. 1997, Chen, Lee et al. 2003, Siwinska, Mielzynska et al. 2004). As in the papers cited above, we saw that occupational exposure in these workers overshadowed any effect of smoking including cigarettes per day. Job duration had an inverse effect on 1HP levels especially in oven workers, suggesting that more senior workers removed themselves from the jobs or tasks having highest exposure.

We also report that the levels a carcinogen-DNA adduct (Adduct 4) were significantly increased in oven workers compared to supervisors and area workers. Figure 3 compares the Adduct 4 levels for the two groups of workers : We saw that levels of DNA Adduct 4 in oven workers averaged 17.43 adducts per 10^9 nucleotides (se=4.61) while the levels in supervisors and area workers was 3.12 adduct per 10^9 unadducted nucleotides (se= 1.05). This difference was statistically significant at $p<0.02$. Other adducts were not related to these 2 exposure groups.

We then investigated the relationship between 1HP levels and the levels of the DNA adducts we saw in the samples from the workers. 1-HP was correlated significantly with DNA adduct 4, suggesting that PAH exposure was responsible for this adduct in exfoliated urothelial cells or that another compound exposure profile was very similar to that of PAH. In our initial analysis we investigated the average levels of DNA Adduct 4 by 1HP quartile values comparing the lowest 1HP quartile to the highest. The results as shown in figure 4. The mean value in the lowest quartile was 1.56 adducts per 10^9nucleotides (se=0.53) and in the highest was 29.97adduct per 10^9nucleotides (se=5.46). This difference was statistically significant at $p<0.01$. Figure 5 shows the relationship between 1HP levels and DNA adduct 4 for all workers. The correlation coefficient was 0.692, which was statistically significantly different from zero ($p<0.0001$). The linear coefficients indicate that the DNA adduct 4 levels at 1 ug/l urine (the ACGIH BEI) would be 3.18 adduct per 10^9 nucleotides. This level is twice as high as the average for workers in the lowest quartile of 1HP levels and suggests that there is the potential for increased genetic damage in even these workers with the lowest exposure in coke plants. We are preparing these data for publication. A preliminary form of this work was presented as an abstract at the NIOSH NORA Manufacturing Sector Council Sector Conference: Partnerships to Improve Occupational Safety and Health, September 7-8, 2011. Polycyclic Aromatic Hydrocarbon Exposure in German Coke Oven Workers (authors:Thoroman, Jeffrey S, Kafferlein, Heiko U., Schumann, Brenda L., Talaska, Glenn). Another abstract, submitted to the 9th International Conference on Biological Monitoring which will be held in Manchester England, September 7-10, 2013, will feature the entire data set.

As the grant progressed we saw that we would be completing all the analysis on the coke oven workers and requested that we be allowed to add data from analysis of firefighters to the coke oven worker database. We felt that the exposure of the coke oven workers was at the high end

of PAH exposed workers and we believed that we could get more useful data to address the relationship between DNA adducts and PAH internal dose from workers with lower exposure levels. These analyses have not been completed although we will complete them in the near future and will forward our progress (and acknowledge the support from this NIOSH grant) as the data are obtained and analyzed.

Regarding specific aim 2 we proved in principle that we could develop a sensor for the real time measurement of 1-hydroxypyrene. Spectroelectrochemical sensing in an optically transparent thin layer electrode (OTTLE) cell was used for detecting the polycyclic aromatic hydrocarbon (PAH) biomarkers 1-hydroxypyrene (1-pyOH) and 1-hydroxypyrene-glucuronide (1-pyOglu) in phosphate buffer and artificial urine. This approach uses selective electrochemical modulation of a fluorescence signal by sequentially oxidizing the analytes in an OTTLE cell to distinguish between their overlapping fluorescence spectra. This technique allows for complete oxidation and signal modulation in approximately 15 min for each analyte; a mixture of 1-pyOH and its glucuronic acid conjugate can be analyzed in 30 min. Calibration curves consisting of the fluorescence change vs analyte concentration for 1-pyOH and 1-pyOglu yielded linear ranges from 10 nM to 1 μM and from 1 nM to 1 μM, respectively. With the use of these results, the calculated limits of detection were determined to be 1 _ 10_8 M for 1-pyOH and 9 _ 10_11 M for 1-pyOglu.

This work was published: **Wilson, R.A., C.J. Seliskar, G. Talaska, and W.R. Heineman:** Spectroelectrochemical sensing of pyrene metabolites 1-hydroxypyrene and 1-hydroxypyrene-glucuronide. *Analytical Chemistry* 83(10): 3725-3729 (2011).

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