

Albumin Adducts of Naphthalene Metabolites as Biomarkers of Exposure to Polycyclic Aromatic Hydrocarbons

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Abstract

We investigated the utility of adducts formed by the reaction of the naphthalene metabolites naphthalene-1,2-oxide, 1,2-naphthoquinone (1,2-NPQ), and 1,4-naphthoquinone (1,4-NPQ) with serum albumin (Alb) as biomarkers of exposure to polycyclic aromatic hydrocarbons. Cysteinyl serum Alb adducts of 1,2- and 1,4-NPQ (1,2-NPQ-Alb and 1,4-NPQ-Alb, respectively) but not of naphthalene-1,2-oxide were detected in 28 coke oven workers and 22 controls from the steel industry of northern China. The median level of 1,2-NPQ-Alb in coke oven workers (76.6 pmol/g) was significantly higher than that observed in controls (44.9 pmol/g; $P = 0.0027$). However, the median level of 1,4-NPQ-Alb in exposed subjects was not significantly different from that of controls (48.6 versus 44.2 pmol/g; $P = 0.296$). Levels of 1,2-NPQ-Alb were significantly correlated with exposure category (controls, side and bottom workers, and top-of-oven workers) as well as with previously measured levels of urinary naphthalene, 1- and 2-naphthol, and 1-pyrenol in these subjects. Multiple linear regression analysis revealed that 35% of the variation in 1,2-NPQ-Alb could be explained by the work category and age. A negative relationship between 1,2-NPQ-Alb and age was observed, suggesting that cytochrome P450 *c* metabolism diminished with age at ~3%/year of life.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) represent a complex mixture of chemicals, some of which have been recognized as cytotoxic and carcinogenic in humans (1–4). Because PAHs are abundant in many petroleum and coal-derived products, such as kerosene, diesel and jet fuels, coal tar, and coke, workers are exposed to PAHs in industries that produce, process, or con-

sume these fuels (1, 2, 5–7). Exposures to PAHs are greatest in the aluminum, iron, and steel industries (1, 7, 8). Major sources of environmental exposure to PAHs are from inhalation of engine exhaust and tobacco smoke, cooking fires, and from ingestion of smoked foods (9, 10).

Most human studies using biological monitoring of PAHs have measured urinary 1-pyrenol, the most prominent metabolite of pyrene, a four-ring PAH. Indeed, urinary 1-pyrenol has been shown to be a consistent and reliable biomarker of PAH exposure in occupational populations (reviewed in Refs. 11–13). However, urinary biomarkers, such as 1-pyrenol, are eliminated rapidly from the body and hence reflect only short-term exposures of ≤ 1 day. Because exposures vary greatly from day to day, many measurements of these short-term biomarkers are needed to characterize chronic exposures of relevance to epidemiology. Thus, it would be useful to have reliable long-term biomarkers of PAH for use in epidemiology studies.

The only intermediate/long-term biomarkers of PAH reported to date in occupational studies have been DNA and protein adducts of various PAH-derived binding species. Of these, most attention has focused on DNA adducts measured by either ³²P-postlabeling or ELISA, two methods of limited specificity (reviewed in Refs. 12, 13). This lack of specificity, along with inadequate exposure assessment, has probably contributed to the inconsistent comparisons of DNA adducts with personal PAH exposure. For example, mean levels of PAH-DNA adducts measured by ³²P-postlabeling covered a roughly 10-fold range in three studies of iron foundry workers exposed to equivalent air levels of benzo(*a*)pyrene, a five-ring PAH (14–16).

In humans, stable hemoglobin and serum albumin (Alb) adducts have respective residence times in blood of 4 months and 1 month (17). A few studies have investigated PAH-derived adducts of hemoglobin and Alb in worker populations, and these also have shown inconsistent results (possibly because most used ELISA for quantitation; Refs. 18–21). Apparently the only study to measure specific PAH-protein adducts was that of Ferreira *et al.* (22) and Tas *et al.* (20), who reported significant correlations between hemoglobin and Alb adducts, respectively, of benzo(*a*)pyrene diol epoxide with personal exposures to benzo(*a*)pyrene in a steel foundry and a graphite-electrode-production facility.

The purpose of the current investigation was to determine whether protein adducts derived from naphthalene, the simplest PAH, might be useful biomarkers of intermediate/long-term exposure to PAHs. Unlike benzo(*a*)pyrene and the other particulate-phase PAHs, whose abundance represents, at most, a small percentage of the mixture, naphthalene is generated in the gas phase and tends to be the most abundant PAH released from a given source (23–25). We therefore hypothesized that naphthalene-derived adducts would be relatively abundant in workers routinely exposed to PAHs.

As shown in Fig. 1, naphthalene is metabolized by cyto-

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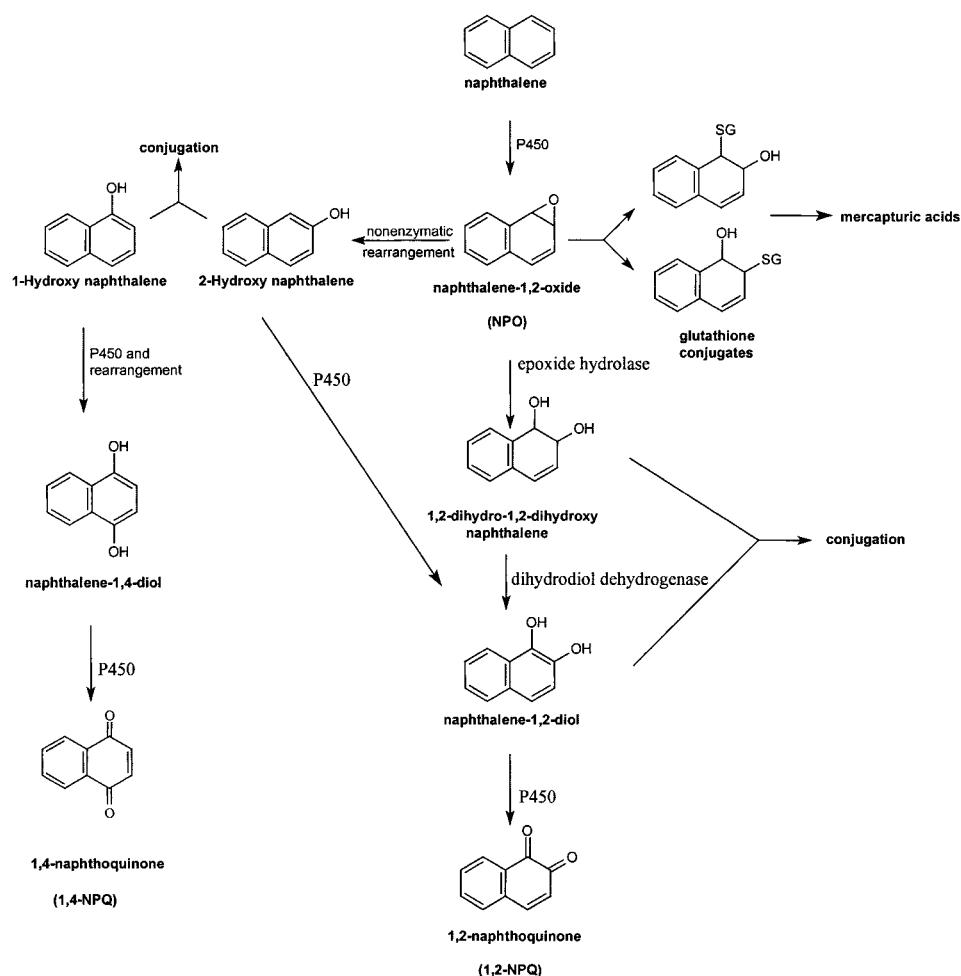


Fig. 1. Proposed metabolic pathway of naphthalene leading to the formation of major metabolites.

chrome P450 isozymes (CYP1A1, -1A2, -2A1, -2E1, and -2F2) to naphthalene-1,2-oxide (NPO) and subsequently to 1,4-naphthoquinone (1,4-NPQ) and 1,2-naphthoquinone (1,2-NPQ; Refs. 26, 27), all of which can form macromolecular adducts with proteins and DNA (28–33). We have shown previously that NPO, 1,2-NPQ, and 1,4-NPQ all bind in a dose-dependent manner to cysteinyl residues in hemoglobin and Alb in F344 rats that had been administered naphthalene (28). The assay used for that study (designated the MT assay) used methanesulfonic acid and trifluoroacetic anhydride (TFAA) to cleave cysteinyl adducts from the proteins while simultaneously converting them to volatile derivatives (see Fig. 2) for quantitation by gas chromatography–negative-ion chemical ionization–mass spectrometry. In this study, we used the MT assay to measure cysteinyl Alb adducts of NPO and the NPQs in 28 coke oven workers and 22 concurrent controls from the steel industry of northern China. We recently showed that levels of urinary products of naphthalene were highly correlated with those of urinary 1-pyrenol in these same workers (34, 35).

Materials and Methods

Chemicals. Standards and internal standards for the assay were prepared as described previously (36). Human Alb, methanesulfonic acid, and hexane (pesticide grade) were from Sigma-Aldrich Inc. (St. Louis, MO), Fluka Chemical Corp.

(Milwaukee, WI), and Fisher Scientific (Pittsburgh, PA), respectively. TFAA was purchased from Pierce (Rockford, IL) and was distilled once before use. (Use caution because TFAA reacts violently with water and should be used only with samples that are completely dry.)

Subjects and Blood Collection. Five ml of venous blood were obtained in heparinized tubes at the end of a 4-day (8 h/day) workweek from 28 coke oven workers (2 women and 26 men, 17 of whom were smokers) from a steel-producing complex in northern China and from 22 office and hospital workers (3 women and 19 men, 3 of whom were smokers) from the same geographical area. Plasma was separated from red cells by centrifugation. Of the coke oven workers, 15 were top-of-oven and 13 were side- or bottom-of-oven workers. The age range for the control subjects (18–50 years) was similar to that of the coke oven workers (29–51 years). Environmental measurements of PAHs were available as benzene-soluble matter from an occupational surveillance program conducted at the coke ovens between 1986 and 1990. Operating conditions and work practices during this period were similar to those at the time of blood sampling (June 2000). On the basis of these measurements, top-of-oven workers had much higher exposures to benzene-soluble matter (mean, 1.37 mg/m³) than side- and bottom-of-oven workers (0.27 and 0.18 mg/m³, respectively). All plasma samples were maintained at –80°C for up to 15

months before analysis. Data on cigarette smoking were obtained by questionnaire.

Measurement of Adducts. Alb was isolated from plasma as reported previously (36). Cysteinyll adducts of 1,2- and 1,4-NPQ were measured as described by Waidyanatha *et al.* (28). Briefly, to 5 mg of protein, 5 μ g of isotopically labeled protein-bound internal standards ($[^2\text{H}_5]$ 1,4-NPQ-Alb, $[^2\text{H}_5]$ 1,2-NPQ-Alb, $[^2\text{H}_7]$ NPO1-Alb, and $[^2\text{H}_7]$ NPO2-Alb) were added, and samples were brought to dryness in a vacuum oven (70–80°C at 15 mm Hg). The dried proteins were reacted with 750 μ l of TFAA and 20 μ l of methanesulfonic acid at 100°C for 40 min to produce 1,2-NPQ-S-TFA, 1,4-NPQ-S-TFA, NPO1-S-TFA, and NPO2-S-TFA from 1,2-NPQ-Alb, 1,4-NPQ-Alb, NPO1-Alb, and NPO2-Alb, respectively (Fig. 2). Samples were cooled to room temperature, and unreacted TFAA was removed under a gentle stream of N_2 . One ml of hexane was added to the residue, and the hexane layer was washed once with 1 ml of 0.1 M Tris buffer (pH 7.3) and twice with 1 ml of deionized water, after which it was concentrated to 100 μ l. Three- μ l aliquots were analyzed by gas chromatography–negative-ion chemical ionization–mass spectrometry using a HP 5890 series II gas chromatograph coupled to a HP 5989B MS engine. A DB-5 fused-silica capillary column (60 m long; 0.25 mm i.d.; 0.25- μ m film thickness) was used with helium as the carrier gas at a flow rate of 1.5 ml/min; the injection-port temperature was 250°C. The MS transfer-line temperature was 280°C, and the chemical ionization reagent gas (methane) pressure was 2 Torr. For the quantitation of 1,2- and 1,4-NPQ-Alb, the oven temperature in the gas chromatograph was held at 75°C for 2 min, then increased at 6°C/min to 150°C, where it was held for 28

min. For quantitation of NPO-Alb, the oven temperature was held at 75°C for 2 min and increased at 4°C/min to 160°C, where it was held for 15 min. Late-eluting compounds were removed by increasing the oven temperature at 50°C/min to 260°C, where it was held for 15 min. The following ions were monitored: m/z 383 for 1,2- and 1,4-NPQ-S-TFA; m/z 388 for $[^2\text{H}_5]$ 1,2- and 1,4-NPQ-S-TFA; m/z 256 for NPO1- and NPO2-S-TFA; and m/z 263 for $[^2\text{H}_7]$ NPO1- and $[^2\text{H}_7]$ NPO2-S-TFA. The quantitation was based on peak areas relative to the internal standards.

On the basis of a signal-to-noise ratio of 3, when 5 mg of Alb were used, the limit of detection of this assay corresponded to ~ 10 pmol adduct/g of Alb.

Statistical Analysis. The coefficients of variation (CVs) of the assay for 1,2- and 1,4-NPQ-Alb and for injections into the gas chromatograph–mass spectrometer were estimated from the variance components obtained with a nested random-effects model (injections nested within assays) by Proc NESTED of SAS using the log-transformed levels of adducts (37). The data for these analyses were obtained by assaying 20 duplicate samples from coke oven workers and controls and by randomly re-injecting 24 samples. From this, the CV representing injection error is reported as $\text{CV}_i = \sqrt{e^{s_e^2} - 1}$, where s_e^2 is the estimated error variance component. Assay error is reported as $\text{CV}_a = \sqrt{e^{s_w^2} - 1}$, where s_w^2 is the estimated within-subject variance component (37). The CV representing the overall method (combined assay plus injection errors) is given by $\text{CV}_m = \sqrt{e^{(s_e^2 + s_w^2)} - 1}$.

Subjects were divided into three exposure categories: controls, side- and bottom-of-oven workers, and top-of-oven work-

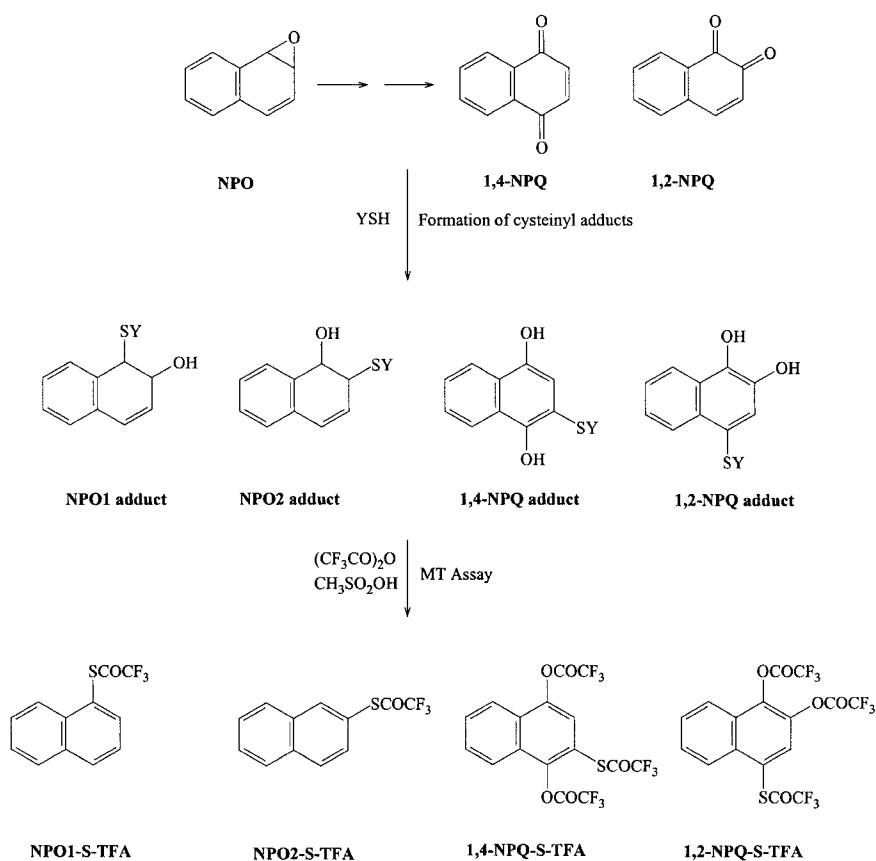


Fig. 2. Formation of cysteinyl adducts of naphthalene oxide (NPO) and 1,2- and 1,4-naphthoquinone (1,2-NPQ and 1,4-NPQ) and subsequent reaction of these adducts with trifluoroacetic anhydride and methanesulfonic acid (MT assay) to produce trifluoroacetylated (TFA) derivatives for detection by gas chromatography–negative-ion chemical ionization–mass spectrometry.

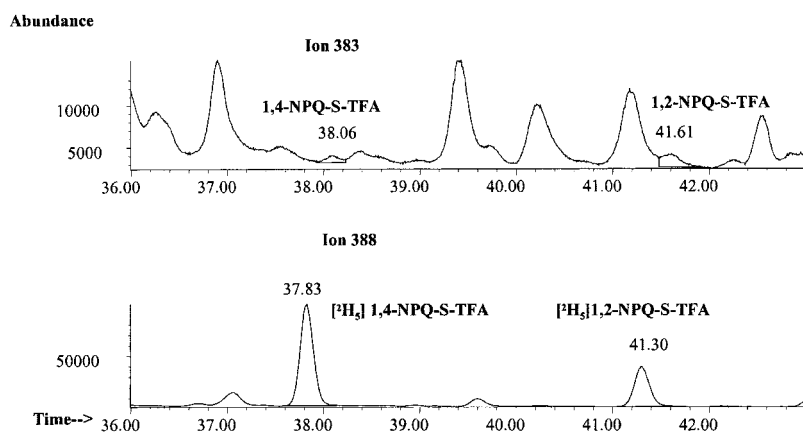


Fig. 3. Typical gas chromatography-negative-ion chemical ionization-mass spectrometry chromatogram obtained in selected-ion monitoring mode after reaction of 5 mg of albumin from a top-of-oven worker with trifluoroacetic anhydride and methanesulfonic acid. The ion m/z 383 corresponds to trifluoroacetyl (TFA) derivative of 1,2- and 1,4-naphthoquinone (1,2-NPQ and 1,4-NPQ) adducts. The corresponding deuterated internal standard ion was m/z 388.

ers. The Wilcoxon rank-sum test was used to evaluate differences in protein adduct levels between exposed and control subjects, between smokers and nonsmokers in each exposure category, and between the particular adducts. The correlations of adduct levels among exposure categories were evaluated using Spearman coefficients (r_s).

In light of the highly skewed distributions, all other statistical analyses were carried out using (natural) logarithmic transformation. Linear correlations were investigated between individual (logged) adduct levels, previously reported (logged) levels of urinary naphthalene, and (logged) levels of urinary naphthols and 1-pyrenol by use of Pearson coefficients (r). Multiple linear regressions (Proc REG of SAS) were conducted to evaluate the impact on levels of Alb adducts of the following variables: work category (using dummy variables), age, gender, years of coke oven work, cigarette smoking, and levels of urinary naphthalene and 1- and 2-naphthol. Backward elimination was used with retention of variables at a $P < 0.1$. Residuals were visually inspected for normality by use of q - q plots and were investigated for influence and possible outliers based on Studentized residuals, leverage, and Cook's distance. No observations were rejected based on these criteria.

All statistical analyses were performed with SAS system software (V. 8.1; SAS Institute, Cary, NC) with the significance level set at 0.05 (two-tailed).

Results

1,2- and 1,4-NPQ Adducts in Coke Oven Workers and Controls. Both 1,2- and 1,4-NPQ adducts were detected in all exposed and control subjects. However, isomers of NPO adducts were not detected in any subject. The precision (CV_m) of the method for 1,2- and 1,4-NPQ-Alb, respectively, based on 28 duplicate assays and 24 duplicate injections into the gas chromatography-mass spectrometer was 47% and 55%, representing the combined injection ($CV_i = 25\%$ and 20%) and assay ($CV_a = 40\%$ and 51%) errors. A typical gas chromatography-negative-ion chemical ionization-mass spectrometry chromatogram (in selected-ion monitoring mode) is shown in Fig. 3, which shows the analysis of Alb from a top-of-oven worker.

Summary statistics of the study population and NPQ adducts are given in Table 1 by exposure category (controls, side- and bottom-of-oven workers, and top-of-oven workers) and smoking status. The median level of 1,2-NPQ-Alb in coke oven workers (76.6 pmol/g) was significantly higher than that observed in controls (44.9 pmol/g; $P = 0.0027$). However, the median level of 1,4-NPQ-Alb in exposed subjects was not significantly different from that of controls (48.6 versus 44.2 pmol/g; $P = 0.296$). No significant differences were detected in median adduct levels between smokers and nonsmokers in any exposure category. As shown in Fig. 4, there was a significant positive correlation between median levels of 1,2-NPQ-Alb and

Table 1 Summary of albumin adducts of 1,2- and 1,4-NPQ^a among coke oven workers by work category (controls, side- or bottom-of-oven workers, and top-of-oven workers) and smoking status

	Exposure category					
	Controls		Side- or bottom-of-oven workers		Top-of-oven workers	
	Smokers (n = 3)	Nonsmokers (n = 19)	Smokers (n = 8)	Nonsmokers (n = 5)	Smokers (n = 9)	Nonsmokers (n = 6)
1,2-NPQ-Alb (pmol/g Alb)						
Mean ± SD	56.1 ± 11.7	61.5 ± 54.3	76.2 ± 40.8	68.6 ± 24.5	117 ± 61.9	98.6 ± 41.5
Median	55.3	44.2	70.9	62.6	102	95.6
Range	44.7-68.1	11.4-194	19.7-132	40.4-108	47.5-227	50.6-150
1,4-NPQ-Alb (pmol/g Alb)						
Mean ± SD	60.6 ± 43.9	44.9 ± 17.3	49.4 ± 16.0	47.9 ± 19.4	62.3 ± 50.4	80.5 ± 60.3
Median	36.1	44.9	48.6	46.0	49.9	59.4
Range	34.4-111	19.3-69.9	31.8-78.5	27.6-74.7	29.2-194	30.3-186

^a NPQ, naphthoquinone; Alb, albumin.

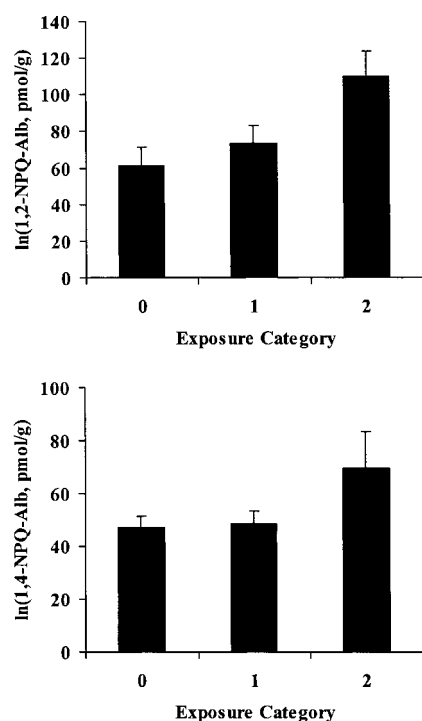


Fig. 4. Effect of exposure category (0, office and clerical workers; 1, side- and bottom-of-oven workers; 2, top-of-oven workers) on levels of 1,2- and 1,4-naphthoquinone (1,2-NPQ-Alb and 1,4-NPQ-Alb). Each column represents the mean (SE; error bars). On the basis of the historical measurements of benzene-soluble matter (BSM), the top-of-oven workers were exposed to 1.37 mg BSM/m³, side-of-oven workers to 0.27 mg BSM/m³, and bottom-of-oven workers to 0.18 mg BSM/m³.

exposure category ($r_s = 0.496$; $P = 0.0003$) but not for similar comparisons involving 1,4-NPQ-Alb ($r_s = 0.173$; $P = 0.230$).

When analyzed on an individual basis (Table 2), logged levels of 1,2-NPQ-Alb were significantly correlated with 1,4-NPQ-Alb ($r = 0.361$; $P = 0.010$). Because we had previously measured urinary levels of naphthalene, 1- and 2-naphthols, and 1-pyrenol in the same subjects, the correlations between adducts and these urinary biomarkers were also investigated. 1,2-NPQ-Alb showed significant correlations with all urinary products (naphthalene, 1- and 2-naphthol, and 1-pyrenol; $r \geq 0.371$; $P \leq 0.0081$), as indicated by the scatter plots in Fig. 5. When the corresponding comparisons were investigated with 1,4-NPQ-Alb, no significant correlations were observed for any of the urinary products ($r \leq 0.241$; $P \geq 0.083$; Table 2).

The contributions to levels of 1,2- and 1,4-NPQ-Alb from work category, age, gender, years of coke oven work, smoking status, and urinary levels of naphthalene and 1- and 2-naphthols were estimated by multiple linear regression. As shown in Table 3, the final model for 1,2-NPQ-Alb included only work category and age. On the basis of the ΔR^2 values, work category explained ~28% of the variability of levels of this adduct (10.1% for side- and bottom-of-oven worker and 18.3% for a top-of-oven workers), and age explained ~6%. Thus, the explained variability (R^2) of levels of 1,2-NPQ-Alb was 0.34. The parameter estimate for age was negative, implying that levels of 1,2-NPQ-Alb decreased with increasing age. For 1,4-NPQ-Alb, significant contributions to adduct levels were observed for work category and for 1- and 2-naphthol ($R^2 = 0.18$; Table 3). Although the parameter estimates for 2-naphthol were positive,

indicating that 1,4-NPQ-Alb increases with increasing 2-naphthol, the reverse was true for 1-naphthol.

Discussion

The utility of naphthalene-derived Alb adducts as intermediate/long-term biomarkers of exposure to PAHs was investigated in 28 coke oven workers and 22 concurrent controls from the steel industry in northern China. Adducts of both 1,2- and 1,4-NPQ were detected in all subjects. Lower assay precision was observed in this study ($CV_m = 47\%$ and 55% for 1,2-NPQ-Alb and 1,4-NPQ-Alb, respectively) compared with that reported previously for these adducts using *in vitro* modified proteins ($CV_m = 13\%$ and 18% ; Ref. 28) and for Alb adducts of 1,4-benzoquinone ($CV_m = 20\%$; Ref. 38). This probably reflects difficulties in quantifying the lower levels of adducts in the present study compared with those reported previously.

In controls, the median level of 1,2-NPQ-Alb was similar to that of 1,4-NPQ-Alb (44.9 versus 44.2 pmol/g). However, in exposed subjects, the median level of 1,2-NPQ-Alb was significantly higher than that of 1,4-NPQ-Alb (76.6 versus 48.6 pmol/g; $P = 0.0008$). We previously observed higher levels of 1,2-NPQ-Alb than 1,4-NPQ-Alb in F344 rats administered with naphthalene intragastric (0–800 mg/kg of body weight; Ref. 28). The greater abundance of the 1,2-NPQ adduct could reflect the presence of multiple pathways for the formation of 1,2-NPQ but not of 1,4-NPQ (see Fig. 1) as well as differences in the relative rates of formation and removal of the respective adducts *in vivo* (29).

Adducts of NPO with Alb were not observed in our study, indicating that levels of NPO-Alb were below the detection limit of the assay (10 pmol/g). This finding was unexpected because we had previously observed that NPO-Alb was more abundant and more stable than 1,2- and 1,4-NPQ-Alb in F344 rats after administration of naphthalene (29). Our inability to detect NPO-Alb is also perplexing in light of previous studies, which reported Alb adducts of epoxides of other aromatic species, notably styrene and benzene, in both occupationally exposed and unexposed subjects (38–41).

Levels of Alb adducts of both 1,2-NPQ and 1,4-NPQ were elevated in coke oven workers compared with controls. However, the effect was not significant for 1,4-NPQ-Alb, perhaps because of reduced precision of the method for this adduct or higher background levels of 1,4-NPQ coming from other sources. Among exposed subjects, the highest adduct levels were observed in top-of-oven workers (Table 1). This is consistent with historical measurements of airborne benzene-solu-

Table 2 Correlation matrix for urinary biomarkers and albumin adducts for coke oven workers ($n = 28$) and control subjects ($n = 22$)

Pearson correlation coefficients and P s are shown for each comparison, based on log-transformed data.

	1-Naphthol	2-Naphthol	1-Pyrenol	1,2-NPQ-Alb ^a	1,4-NPQ-Alb
Naphthalene	0.585 <0.0001	0.603 <0.0001	0.563 <0.0001	0.371 0.0081	0.241 0.0915
1-Naphthol		0.911 <0.0001	0.876 <0.0001	0.390 0.0051	0.031 0.8296
2-Naphthol			0.857 <0.0001	0.479 0.0003	0.138 0.3367
1-Pyrenol				0.377 0.0069	0.083 0.564
1,2-NPQ-Alb					0.361 0.0100

^a NPQ, naphthoquinone; Alb, albumin.

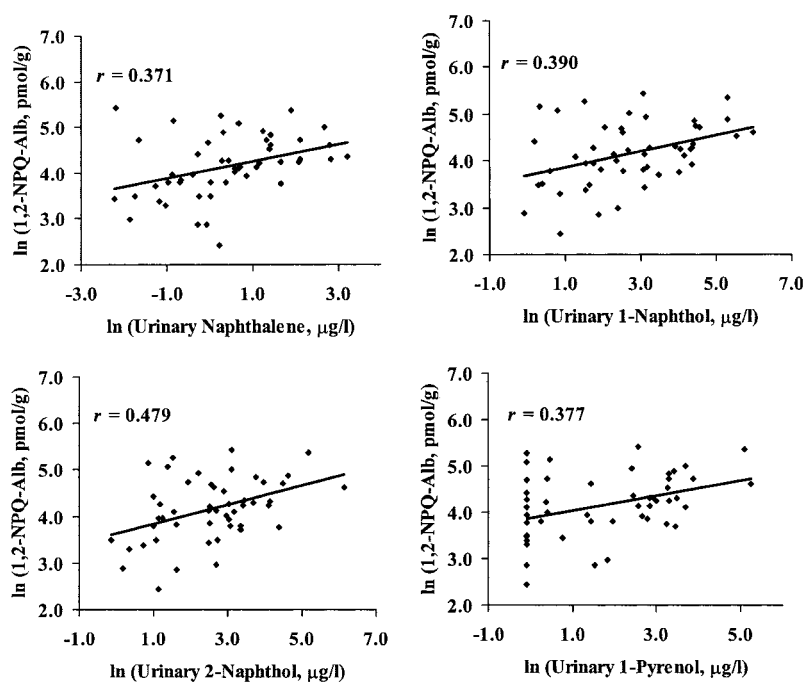


Fig. 5. Scatter plots of 1,2-naphthoquinone-albumin adducts (1,2-NPQ-Alb) versus previously measured urinary levels of naphthalene, 1- and 2-naphthol, and 1-pyrenol in 28 coke oven workers and 22 controls. Lines represent simple linear regression given by $\ln(1,2\text{-NPQ-Alb}) = 4.08 + 0.185 \ln(\text{naphthalene})$; $\ln(1,2\text{-NPQ-Alb}) = 3.69 + 0.170 \ln(1\text{-naphthol})$; $\ln(1,2\text{-NPQ-Alb}) = 3.64 + 0.204 \ln(2\text{-naphthol})$; and $\ln(1,2\text{-NPQ-Alb}) = 0.142 + 0.159 \ln(1\text{-pyrenol})$.

ble matter in the Chinese coke ovens as well as with previous reports that top-of-oven workers consistently had the highest exposure to PAHs (42, 43).

As summarized in Table 4, relatively few studies have used blood protein adducts as biomarkers of exposure to PAHs. Ferreira *et al.* (22) observed a significant but weak correlation of hemoglobin adducts of benzo(*a*)pyrene diol epoxide with external exposure (measured as the sum of 13 PAHs) in workers in a steel foundry and graphite electrode production plant ($r = 0.26$; $P = 0.0002$). Three studies measured Alb adducts of PAHs by ELISA in iron foundry workers and controls (18, 19, 21); of these studies, Sherson *et al.* (18) and Lee *et al.* (21) reported group differences in adduct levels between exposed and control subjects that were at or near statistical significance (Table 4). In a much larger study, Tas *et al.* (20), who used high-performance liquid chromatography with fluorescence detection, reported significant correlations on an individual basis between Alb adducts of benzo(*a*)pyrene diol epoxide and benzo(*a*)pyrene exposure ($r = 0.30$; $P = 0.0001$) as well as total PAH exposure ($r = 0.35$; $P = 0.0001$) in workers from a steel foundry and a plant producing graphite electrodes.

Our study departed from earlier investigations of PAH-Alb adducts by investigating adducts arising from the volatile

constituent, naphthalene, rather than those of benzo(*a*)pyrene and other particulate constituents. We found that Alb adducts of 1,2-NPQ were significantly elevated in coke oven workers compared with controls and that the levels of adducts grouped by work category increased in the *a priori* expected order for PAH exposure, *i.e.*, control workers < bottom- and side-of-oven workers < top-of-oven workers. Thus, despite the small sample sizes in our study and the lack of environmental PAH measurements, our results are consistent with the hypothesis that naphthalene-derived adducts reflect the PAH exposures received by coke oven workers.

Moderate but significant correlations were observed between levels of 1,2-NPQ-Alb and all of the urinary biomarkers of naphthalene (naphthalene and 1- and 2-naphthol) and pyrene (1-pyrenol; $r > 0.371$; $P < 0.0081$; Table 2). These moderate correlations are not surprising because urinary measurements were based on a single spot-urine sample that reflected only recent exposure, whereas Alb adducts were presumably integrated over a period of ~30 days (the mean residence time of human serum Alb). The magnitude of these correlations with urinary biomarkers of naphthalene are of the same magnitude as those reported by Tas *et al.* (20) for comparisons between Alb adducts of benzo(*a*)pyrene diol epoxide and personal PAH

Table 3 Coefficients and R^2 values for the final regression models of 1,2- and 1,4-NPQ-Alb^a for 28 coke oven workers and 22 control subjects

Response variable	Overall R^2 (P)	Predictor variable	Coefficients (P)	ΔR^2
1,2-NPQ-Alb	0.346 (0.0002)	Intercept	4.91 (<0.0001)	
		Top-of-oven worker	0.893 (<0.0001)	0.183
		Side- or bottom-of-oven worker	0.570 (0.0105)	0.101
		Age	-0.031 (0.0051)	0.062
1,4-NPQ-Alb	0.176 (0.0289)	Intercept	3.79 (<0.0001)	
		Top-of-oven worker	0.416 (0.0201)	0.062
		1-Naphthol	-0.276 (0.0151)	0.019
		2-Naphthol	0.270 (0.0255)	0.095

^a NPQ, naphthoquinone; Alb, albumin.

Table 4 Summary of PAH^a exposures and protein adducts in occupational studies

Source of exposure	Subjects (n)	Measurement (method)	Results	Reference
Iron foundry	Exposed (45) Controls (45)	PAH-Alb (ELISA)	Controls vs. exposed $P = 0.05$ (smokers) $P = 0.005$ (nonsmokers)	Sherson <i>et al.</i> (19)
Iron foundry and roofers	Exposed (37) Controls (37)	PAH-Alb (ELISA)	Controls vs. exposed $0.05 < P < 0.10$	Lee <i>et al.</i> (21)
Steel foundry and graphite electrode production	Exposed (206)	B(a)PDE-Hb (HPLC-fluorescence)	$r = 0.26$; $P = 0.0002$ (with PAH levels in air)	Ferreira <i>et al.</i> (22)
Iron foundry	Exposed (70) Controls (68)	PAH-Alb (ELISA)	Controls vs. exposed $P = 0.65$ (smokers) $P = 0.20$ (nonsmokers)	Omland <i>et al.</i> (18)
Steel foundry and graphite electrode production	Exposed (127) Controls (133)	B(a)PDE-Alb (HPLC-fluorescence)	$r = 0.35$; $P = 0.0001$ (with PAH in air)	Tas <i>et al.</i> (20)
Steel industry	Exposed (28) Controls (22)	1,2-NPQ-Alb (GC-MS)	$r = 0.496$; $P = 0.0003$ (with exposure category)	Present study

^a PAH, polycyclic aromatic hydrocarbon; PAH-Alb, albumin adducts of total polycyclic aromatic hydrocarbons; B(a)PDE-Hb, hemoglobin adducts of benzo(a)pyrene diol epoxide; HPLC, high-performance liquid chromatography; B(a)PDE-Alb, albumin adducts of benzo(a)pyrene diol epoxide; 1,2-NPQ-Alb, albumin adducts of 1,2-naphthoquinone; GC-MS, gas chromatography-mass spectrometry.

exposure among foundry and graphite-production workers (Table 4). Because 1-pyrenol is generally regarded as the gold standard for short-term occupational exposure to PAHs, the significant correlation of the 1,2-NPQ-Alb with 1-pyrenol offers further promise that 1,2-NPQ-Alb might serve as a useful biomarker of occupational PAH exposure.

Multiple linear regression analysis revealed a weak but significant relationship between 1,4-NPQ-Alb and urinary 1- and 2-naphthol among top-of-oven workers. These three variables explained ~18% of variation of this adduct in these subjects (Table 3). However, the coefficients for 1- and 2-naphthol had opposite signs, indicating that the adduct varied inversely with 1-naphthol and directly with 2-naphthol. This finding is difficult to reconcile with the postulated metabolism of naphthalene (Fig. 1), in which 1-naphthol is the precursor of 1,4-NPQ, and will require additional experimental confirmation. In any case, our results indicate that Alb adducts of 1,2-NPQ are likely to be more useful biomarkers of PAH exposure than those of 1,4-NPQ.

Levels of 1,2-NPQ-Alb were strongly associated with exposure category, with the magnitudes of coefficients favoring the *a priori* expected order noted above (Table 1 and Fig. 4). Furthermore, age was inversely associated with 1,2-NPQ-Alb, indicating that older workers had significantly lower levels of 1,2-NPQ-Alb than younger workers after adjusting for exposure category (Table 3). This supports the idea that the rate of metabolism of naphthalene to 1,2-NPQ was slower in older workers compared with younger workers. On the basis of the coefficient of the (logged) adduct level on age (-0.031), our data point to a 3% reduction of naphthalene metabolism per year of life. This observation is consistent with the results reported by Rappaport *et al.* (38), who reported a 2% reduction of metabolism of benzene to 1,4-benzoquinone, estimated by use of the corresponding Alb adducts in a population of Chinese workers.

It is also worth mentioning that smoking status was not a significant predictor of either 1,2- or 1,4-NPQ-Alb in our study. This is perhaps not surprising because the level of naphthalene in cigarette smoke ($0.276 \mu\text{g}/\text{cigarette}$ in mainstream smoke; Ref. 44) is much lower than the anticipated levels expected in coke oven emissions (42). This result suggests that cigarette smoking is not likely to confound measurements of 1,2-NPQ-Alb, at least among workers with known heavy exposures to PAHs.

Relatively large levels of 1,2- and 1,4-NPQ-Alb (mean, 60.8 and 47.0 pmol/g, respectively) were found among the control subjects in our study (Table 1). These background levels are comparable to levels observed in commercial Alb obtained from Sigma-Aldrich Inc. (St. Louis, MO), representing pooled blood from presumably unexposed persons in the United States (64.0 and 79.0 pmol/g for 1,2- and 1,4-NPQ-Alb, respectively; Ref. 28).

Among the most highly exposed (top-of-oven) workers in our study, no significant increase in the median level of 1,4-NPQ-Alb was observed above that in controls, whereas a 2-fold increase was observed in the level of 1,2-NPQ-Alb above that in controls (Table 1). In the same sample of top-of-oven workers, we previously observed 7–15-fold increases in levels of urinary naphthalene and 1- and 2-naphthol above control levels (28, 34, 35). Therefore, background levels of 1,2- and 1,4-NPQ-Alb in unexposed subjects cannot be explained by environmental exposure to naphthalene alone but rather point to background exposures to the naphthoquinones or naphthalene diols. We previously reported the presence of background adducts of 1,4-benzoquinone (geometric mean, 958 pmol/g), a metabolite of benzene, at levels much higher than those observed for the naphthoquinones in the present investigation (38). The higher levels of benzoquinones suggest greater abundances of benzene and/or phenyl moieties than those of naphthalene in environmental sources such as gasoline, cigarette smoke, and diet (5, 44, 45).

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