Biomarkers, January–February 2006; 11(1): 14–27

Taylor & Francis
Taylor & Francis Group

Variability of albumin adducts of 1,4-benzoquinone, a toxic metabolite of benzene, in human volunteers

Y. S. LIN[1], W. MCKELVEY[1,2], S. WAIDYANATHA[1], & S. M. RAPPAPORT[1]

[1]*Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC 27599, USA and* [2]*Environmental and Occupational Disease Epidemiology, NYC Department of Health & Mental Hygiene, New York, NY 10007, USA*

Abstract

A putative haematotoxic and leukaemogenic metabolite of benzene, 1,4-benzoquinone (1,4-BQ), reacts rapidly with macromolecules. The authors previously characterized levels of the albumin (Alb) adduct (1,4-BQ-Alb) of this reactive species in populations of workers exposed to benzene. Since high levels of 1,4-BQ-Alb were also measured in unexposed workers from those investigations, the current study was initiated to determine potential sources of 1,4-BQ in the general population. A single blood sample was collected from 191 healthy subjects from the Research Triangle area, NC, USA, to determine the baseline 1,4-BQ-Alb levels and contributing sources. The median 1,4-BQ-Alb at baseline was 550 pmol g^{-1} Alb (interquartile range 435–814 pmol g^{-1}). A second blood sample was collected approximately 3 months later from a subgroup of 33 subjects to estimate the within- and between-person variation in 1,4-BQ-Alb. Standardized questionnaires were administered to collect information about demographic, dietary and lifestyle factors. Multiple linear regression models identified several significant contributors to 1,4-BQ-Alb levels, including gender, body mass index (BMI), the gender–BMI interaction, automobile refuelling, smoking status, and consumption of fruit and the artificial sweetener, aspartame. The authors predicted that these background levels of 1,4-BQ-Alb were equivalent to occupational exposures between 1 and 3 parts per million of benzene. Mixed effects linear models indicated that the random variation in adduct levels was about equally divided between and within subjects. The observations indicate that levels of 1,4-BQ-Alb cover a wide range in the general population, and they support the hypotheses that demographic, diet and lifestyle factors are contributing sources.

Keywords: *Background, benzoquinone, albumin adducts, benzene, variance components*

(Received 24 May 2005; accepted 22 September 2005)

Introduction

Benzene, C_6H_6 (CAS No. 71-43-2), is an environmental toxicant present in petroleum and its derivatives, and in organic combustion products, including cigarette smoke (IARC 1989, Wallace 1996). In fact, benzene is ubiquitous in the environment with air levels ranging between ng m^{-3} to μg m^{-3} throughout the world. Occupational exposures to benzene at mg m^{-3} air concentrations has been associated with

Correspondence: S. M. Rappaport. Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC 527599, USA. E-mail: smr@unc.edu

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.1080/13547500500382975

haematotoxicity, including acute myelogenous leukaemia (AML) (Savitz & Andrews 1997, Hayes et al. 2000, Glass et al. 2003, Lan et al. 2004).

Although the mechanism of benzene's haematotoxicity and carcinogenicity are not completely understood, it is accepted that one or more of the reactive metabolites is involved (Snyder 2000, 2002). These reactive metabolites include benzene oxide, 1,2- and 1,4-benzoquinone (1,2-BQ and 1,4-BQ), benzene diolepoxide, and the muconaldehydes. As shown in Figure 1, benzene is transformed by CYP2E1 to benzene oxide-oxepin, which either spontaneously rearranges to phenol or undergoes further metabolism to catechol and the muconaldehydes. Phenol can be acted upon by CYP2E1 to produce hydroquinone, which can subsequently be oxidized, either spontaneously or via peroxidases to 1,4-BQ. It has been increasingly speculated that 1,4-BQ plays a key role in benzene's toxic action due to its intrinsic toxicity to sensitive bone marrow cells, its inhibition of topoisomerase II, and the apparent protective effects of NAD(P)H: quinone reductases (NQO1, which reduces 1,4-BQ to the non-toxic hydroquinone) *in vitro* and *in vivo* (Smith 1999, Lan et al. 2004, Whysner et al. 2004).

Because 1,4-BQ is extremely reactive in the presence of nucleophiles, the present authors studied its disposition in humans using cysteinyl adducts of the blood proteins, haemoglobin (Hb) and albumin (Alb) (Yeowell-O'Connell et al. 2001, Rappaport et al. 2002). Given the apparent role of 1,4-BQ in benzene toxicity, it was a

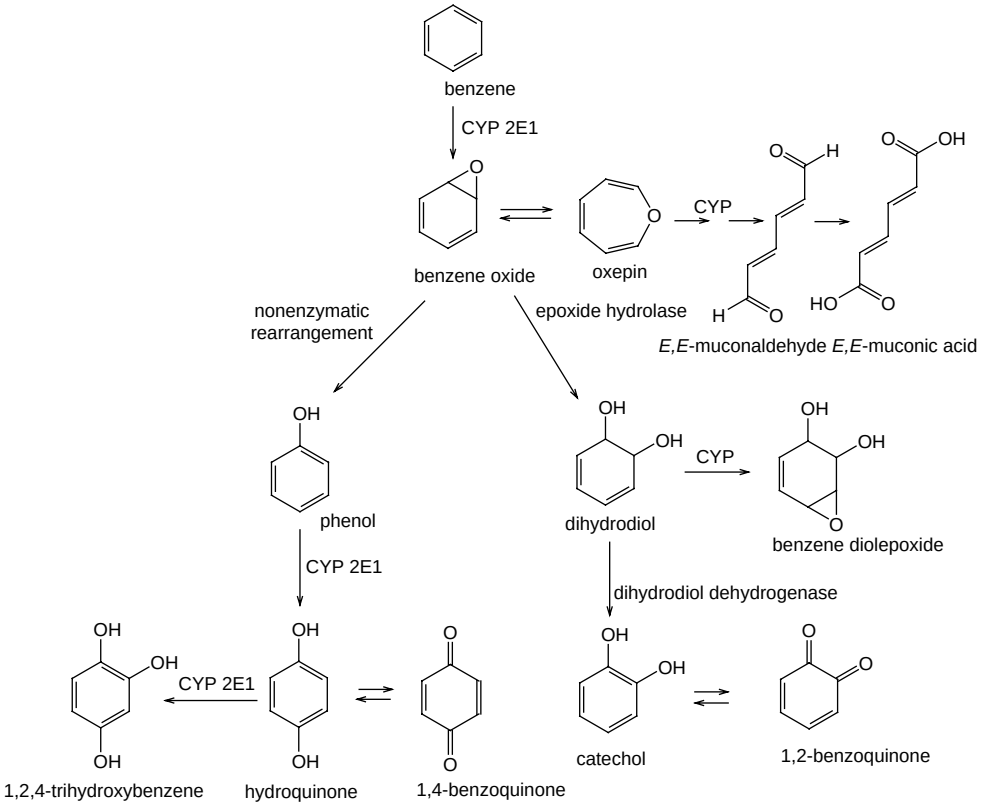

Figure 1. Primary pathways of benzene metabolism. CYP, cytochrome P450.

surprise to discover that the blood of unexposed subjects contained high levels of 1,4-BQ-Alb (McDonald et al. 1993, Yeowell-O'Connell et al. 2001). Indeed, the levels of 1,4-BQ-Alb observed in non-smoking, unexposed workers in Tianjin, China, were equivalent to those of workers exposed to about 6.3 mg m^{-3} (2 ppm) benzene in air (Rappaport et al. 2002), a level twice the current occupational exposure limit in the USA (OSHA 1987). These background adducts point to potential environmental, endogenous and dietary sources of precursors of 1,4-BQ (benzene, phenol and 1,4-hydroquinone), notably cigarette smoke, aromatic amino acids (catabolism of phenylalanine and tyrosine produce phenol), coffee and tea, certain over-the-counter medications (such as Peptol Bismol® and Chloraseptic® lozenges, which contain phenol), and fruits (such as pears) containing large amounts of arbutin, a naturally occurring plant product that is converted to hydroquinone by stomach acid (Deisinger et al. 1996, McDonald et al. 2001). It has been speculated that if 1,4-BQ is truly responsible for the carcinogenicity of benzene, background sources of 1,4-BQ could be associated with much of the incidence of AML in the general population (McDonald et al. 1993, 2001, Smith 1996). With this in mind, the present authors conducted a study of healthy volunteer subjects, stratified by gender, race and smoking habits, to determine how much of the variability in 1,4-BQ levels could be explained by gender, physiological and lifestyle factors, smoking, use of gasoline and other benzene containing products, and diet.

Materials and methods

Study design and subjects

The study was approved by the Human Subject Committee of the Institutional Review Board of the University of North Carolina at Chapel Hill with informed consent obtained at the time of participation. A total of 191 healthy human subjects were recruited from the Research Triangle area of North Carolina during 1998. The goal of this investigation was to recruit approximately balanced numbers of subjects in groups stratified by gender, smoking status and race (Caucasian, African-American). A single blood sample was collected (in heparin) from each subject to determine the level of the cysteinyl albumin adduct of 1,4-BQ-Alb, which represents a measure of the concentrations of free 1,4-BQ in the blood during the previous 2 weeks (Rappaport et al. 2002). An additional blood sample was collected from a subset of 33 subjects, approximately 3 months after the first to characterize the within- and between-person variation in 1,4-BQ-Alb levels. Demographic and dietary characteristics were determined by questionnaires used at the times of recruitment and collection of a second blood sample (if applicable) (see below). All participants were healthy volunteers without known diseases and without known occupational exposures to benzene or petroleum products.

Measurement of 1,4-BQ-Alb

Immediately after phlebotomy, plasma was separated from red cells and the Alb was isolated from plasma and dried to constant weight as described (Lindstrom et al. 1998). After isolation, Alb was stored at −80°C before analysis. Levels of 1,4-BQ-Alb were determined by derivatization and gas chromatography-mass spectrometry during 2002 at the University of North Carolina at Chapel Hill (Waidyanatha et al. 1998).

The estimated limit of detection (LOD) of the assay for 1,4-BQ-Alb was 20 pmol g^{-1} Alb and the precision of the assay (expressed as a coefficient of variation) was 20% (Rappaport et al. 2002).

Evaluation of sources contributing to 1,4-BQ-Alb

Demographic and lifestyle factors. Demographic characteristics, including age and racial background, were obtained with a standardized questionnaire at the time of phlebotomy. Lifestyle information, such as self-service gasoline refuelling (times/week) and daily use of medicine(s) (yes/no), was estimated based on subjects' recall over the past half year. Smoking status was based upon current smoking (yes/no). Consumption of food and beverages was estimated with a dietary questionnaire (see below).

Dietary assessment. A semi-quantitative food-frequency questionnaire (FFQ) containing 131 items was used to evaluate average daily consumption of food, caffeinated beverage consumption and nutrient intake over the past 6 months for each individual (Rimm et al. 1992, Hu et al. 1999). The FFQ used nine categories of consumption frequency, ranging from 'almost never' to 'six or more times/day' for each item. Food consumption was calculated by multiplying the reported frequency by a given serving size of each individual food item, which was combined to compute three major food categories of interest, i.e. vegetables, fruits and total protein (animal, vegetable and plant origin). The FFQ was supplemented with questions regarding the weekly frequency of consumption of fruits and vegetables and monthly consumption of medicines and aspartame, an artificial sweetener derived from phenylalanine (Aspartame Information Center 2005). In addition to total protein, daily consumption of the aromatic amino acids tyrosine and phenylalanine was calculated, since catabolism of these amino acids has been postulated to be a source of phenol and hydroquinone (Peters et al. 1994, Smith & Macfarlane 1996, McDonald et al. 2001). Vitamin intake was calculated by combining intake from both vitamin-containing foods and supplements. All nutrient intake values were compiled at the Channing Laboratory, Harvard Medical School; the validity and reproducibility of the FFQ has been reported (Willett et al. 1985, Salvini et al. 1989, Willett 1998).

Statistical analyses

Association of factors with levels of 1,4-BQ-Alb. The goal was to identify covariates that explained variability in the levels of 1,4-BQ-Alb in non-occupationally exposed subjects. Due to the limited number of persons (33) who provided a second blood specimen, only the baseline data (first blood sample) was used to identify significant covariates and the repeated measurements were only used to estimate within- and between-person variance components. First, non-parametric Spearman correlation coefficients, Wilcoxon rank-sum and Kruskal–Wallis tests were applied to screen the overall and gender-stratified distributions of 1,4-BQ-Alb across demographic, lifestyle and nutrient-intake categories. Then, a multiple regression model was developed using the (natural) log-transformed level of 1,4-BQ-Alb in the ith subject as the dependent variable (logarithmic transformation was needed for uniform variance). Independent variables were chosen from candidates identified in the non-parametric univariate analyses (where $p < 0.15$) and plausible interaction terms. Because the distributions of all continuous variables from this group, except BMI, were highly skewed even after

logarithmic transformation, they were dichotomized, generally using the median. BMI was treated as a continuous variable after (natural) logarithmic transformation. Stepwise selection was used for model building with retention of variables at $p = 0.10$. Normality and linearity were examined using residual plots and multiple collinearity was investigated using condition indices and eigenvalues.

Between- and within-person variation. Between- and within-person variance components for the levels of 1,4-BQ-Alb were estimated with the following linear mixed model:

$$Y_{ij} = \ln(X_{ij}) = \gamma_0 + \beta_i + \varepsilon_{ij} \quad (1)$$

where $i = 1, \ldots, k$ persons and $j = 1, 2$ observations from the ith subject. In model (1), X_{ij} is the level of 1,4-BQ-Alb for the jth observation of the ith person, Y_{ij} is the natural logarithm of X_{ij}, γ_0 is the fixed population mean level of 1,4-BQ-Alb (intercept), β_i is the random effect of the ith person, and ε_{ij} is the random-error effect associated with the jth observation of the ith person. The random effects β_i and ε_{ij} are assumed to be mutually independent and normally distributed with means of zero and variances of σ_B^2 and σ_W^2, representing the between- and within-person variance components, respectively. A compound symmetric variance-covariance structure was assumed and restricted maximum likelihood (REML) estimation was used. The estimates of σ_B^2 and σ_W^2 are designated as $\hat{\sigma}_B^2$ and $\hat{\sigma}_W^2$, respectively, and the estimated intraclass correlation coefficient as:

$$\hat{\rho} = \frac{\hat{\sigma}_B^2}{(\hat{\sigma}_B^2 + \hat{\sigma}_W^2)}.$$

The SAS standard package for Windows version 8.2 (SAS Institute, Inc., Cary, NC, USA) was used for statistical analyses.

Results

Effects of covariates upon adduct levels at baseline

Of the 191 subjects recruited into the study, baseline measurements of 1,4-BQ-Alb were available for 185 subjects, including 94 males and 91 females. (Assays of six subjects were lost due to equipment malfunctions and laboratory mishaps.) Baseline characteristics of 185 subjects are summarized in Table I. Generally, no significant differences were found in medians between males and females in demographic, lifestyle and dietary characteristics. However, medians of body weight, total protein consumption, and intake of tyrosine and phenylalanine were higher for males, and the uses of medications and consumption of tea were higher for females ($p < 0.05$, Wilcoxon rank-sum tests). The daily caloric intake was also higher among males (median 2394 kcal; interquartile range 1924–3133 kcal) than females (median 1938 kcal; interquartile range 1617–2854 kcal) (data not shown).

All 1,4-BQ-Alb measurements at baseline and follow-up were well above the detection limit of 20 pmol g^{-1} Alb (range 206–4716 pmol g^{-1} Alb, $n = 218$). Among the 185 subjects with baseline measurements, the median of 1,4-BQ-Alb was 550 pmol g^{-1} Alb (interquartile range 435–814 pmol g^{-1}). Females had generally greater levels of 1,4-BQ-Alb (median = 662 pmol g^{-1}) than males (median = 498 pmol g^{-1}) ($p < 0.001$, Wilcoxon rank-sum tests).

Table I. Baseline characteristics of 185 human subjects (94 males and 91 females)

Variables	Median (interquartile range)	Range
Physiological factors		
Age (years)	25.2 (22.1–27.7)	18.8–34.5
Body mass index (kg m^{-2})	24.4 (21.6–27.3)	16.9–59.9
Dietary factors:		
Fruit consumption (times/week)	3 (2–7)	0–21
Vegetable consumption (times/week)	4 (2–7)	0–28
Total protein (animal and plant, g day^{-1})	91.1 (63.0–119)	26.6–414
Phenylalanine (g day^{-1})	3.9 (2.7–5.1)	1.1–17.8
Tyrosine (g day^{-1})	3.2 (2.2–4.2)	1.0–14.6
Total vitamin (diet and supplements)		
A (IU day^{-1})*	8550 (5672–13477)	1225–100488
C (mg day^{-1})	229 (127–340)	38.5–1340
E (IU day^{-1})	10.3 (7.1–20.5)	1.6–822
Demographic and lifestyle factors		
Alcohol (drinks/week)†	2.2 (0.2–6.5)	0–73.5
Coffee (cups/day)	0 (0–0.4)	0–4.5
Gas refuelling (times/month)	4.5 (3.5–9)	0–45
Tea (cups/day)	0.1 (0–0.4)	0–6
Artificial sweetener (aspartame) (times/month)	1 (0–12)	0–155
	n (%)	
Current smoking		
Yes	81 (44)	
No	104 (56)	
Education		
High school or less	31 (17)	
Some college or technical school	74 (40)	
College graduate or higher degree	80 (43)	
Ethnicity		
Non-Caucasian‡	88 (48)	
Caucasian	97 (52)	
Use medicine(s) on a monthly basis	98 (53)	

*IU, International unit.
†A drink was 12 of beer, 4 of wine or 1.5 of spirits.
‡Predominatly African American.

Table II summarizes potential determinants of 1,4-BQ-Alb identified by univariate analyses (Wilcoxon rank-sum test or Kruskal–Wallis test with $p < 0.15$). Overall, median levels of 1,4-BQ-Alb were significantly higher among smokers, among subjects with lower BMI, among coffee drinkers, and among subjects who took over-the-counter medicines on a monthly basis ($p < 0.05$). Marginally significant increases ($0.05 \leq p \leq 0.10$) were also observed for subjects who were African-American, or consumed artificial sweetener (aspartame) at least once per month, or refuelled their automobiles more than five times per month, or consumed fruits more than three times per week. Similar monotonic trends were found using the Spearman correlation coefficients. That is, levels of 1,4-BQ-Alb were inversely associated with BMI ($r_S = -0.19$, $p = 0.01$), and positively associated with coffee intake (cups/day) ($r_S = 0.16$, $p = 0.03$), artificial sweetener (aspartame, times/month) ($r_S = 0.15$, $p = 0.04$), gas refuelling (times/month) ($r_S = 0.11$, $p = 0.12$), and fruit consumption

Table II. Baseline levels of 1,4-BQ-Alb (pmol g^{-1} Alb) stratified by demographic, diet and lifestyle characteristics*

	Total			Male			Female		
Variables	*n*	Mean ± SD (median)	*p*	*n*	Mean ± SD (median)	*p*	*n*	Mean ± SD (median)	*p*
Body mass index									
>25 kg m^{-2}	81	637 ± 420 (524)	0.03	45	661 ± 492 (504)	0.36	36	607 ± 314 (542)	<0.001
=25 kg m^{-2}	104	799 ± 601 (610)		49	559 ± 358 (464)		55	1012 ± 691 (816)	
Fruit consumption									
More than three times/week	90	795 ± 615 (589)	0.07	43	681 ± 498 (524)	0.11	47	899 ± 695 (701)	0.59
Less than or equal to three times/week	95	665 ± 439 (509)		51	547 ± 352 (462)		44	802 ± 492 (613)	
Phenylalanine									
>4 g day^{-1}	86	791 ± 611 (577)	0.17	52	655 ± 470 (511)	0.27	34	998 ± 741 (723)	0.07
=4 g day^{-1}	99	674 ± 454 (537)		42	550 ± 367 (473)		57	765 ± 492 (599)	
Alcohol intake									
>2 drinks/week	94	787 ± 616 (590)	0.11	53	626 ± 421 (524)	0.11	41	997 ± 755 (772)	0.08
=2 drinks/week	91	667 ± 430 (527)		41	586 ± 441 (456)		50	733 ± 414 (595)	
Coffee intake (daily)									
Yes	88	783 ± 592 (599)	0.02	47	645 ± 449 (518)	0.10	41	942 ± 694 (729)	0.08
No	97	678 ± 474 (517)		47	572 ± 407 (446)		50	778 ± 513 (595)	
Gas refuelling									
More than five times/month	85	768 ± 496 (602)	0.07	44	654 ± 425 (524)	0.23	41	892 ± 541 (765)	0.16
Less than or equal to five times/month	100	694 ± 565 (528)		50	568 ± 431 (481)		50	819 ± 654 (599)	
Tea intake (daily)									
Yes	113	744 ± 519 (598)	0.12	50	657 ± 465 (527)	0.11	63	813 ± 551 (677)	0.65
No	72	703 ± 561 (509)		44	553 ± 379 (471)		28	939 ± 711 (609)	
Current smoking									
Yes	104	760 ± 532 (589)	0.05	56	629 ± 422 (521)	0.12	48	913 ± 605 (747)	0.08
No	81	687 ± 539 (524)		38	578 ± 440 (453)		43	783 ± 601 (593)	
Artificial sweetener (aspartame) (times/month)									
>1 time/month	84	809 ± 647 (584)	0.09	43	650 ± 474 (537)	0.08	41	976 ± 760 (730)	0.24
=1 time/month	101	661 ± 410 (524)		51	573 ± 386 (446)		50	750 ± 418 (641)	

Table II (*Continued*)

Variables	Total			Male			Female		
	n	Mean ± SD (median)	*p*	*n*	Mean ± SD (median)	*p*	*n*	Mean ± SD (median)	*p*
Ethnicity									
Non-Caucasian‡	88	663 ± 423 (530)	0.07	45	622 ± 445 (456)	0.39	43	707 ± 399 (596)	0.03
Caucasian	97	787 ± 615 (575)		49	596 ± 416 (517)		48	981 ± 721 (763)	
Medicine use (monthly)									
Yes	98	815 ± 622 (595)	0.01	33	620 ± 502 (518)	0.74	65	915 ± 656 (677)	0.11
No	87	630 ± 396 (501)		61	602 ± 386 (466)		26	695 ± 418 (647)	

*Wilcoxon rank-sum text of Kruskal-Wallis test.
‡Predominantly African-American.

Table III. Multiple regression model for log-transformed 1,4-BQ-Alb (pmol g^{-1} Alb) at baseline ($n = 185$)*

Explanatory variables	Parameter estimate	95% CI†	p	$\Delta R^{2‡}$
Intercept	8.860	7.561, 10.16	< 0.001	
Body mass index (BMI; log-transformed, kg m^{-2})	−0.800	−1.205, −0.396	0.001	0.068
Gender (male)	−2.597	−5.131, −0.063	0.045	0.018
Gender (male) * BMI (log-transformed)	0.711	−0.075, 1.496	0.076	0.014
Automobile refuelling (more than five times/month)	0.176	0.023, 0.329	0.025	0.023
Artificial sweetner (aspartame) (more than once a month)	0.141	−0.005, 0.287	0.059	0.016
Current smoking (yes)	0.142	−0.010, 0.293	0.066	0.015
Fruit consumption (more than three times/week)	0.134	−0.013, 0.282	0.074	0.015

*R^2 and adjusted R^2 were 0.21 and 0.18, respectively.
†95% confidence interval for the parameter estimated.
‡Adjusted partial R^2.

(times/week) ($r_S = 0.10$, $p = 0.14$). However, no trend was observed between levels of 1,4-BQ-Alb and phenylalanine intake (g day^{-1}) ($r = 0.02$, $p = 0.82$).

The final multiple regression model is shown in Table III for variables that significantly affected (log-transformed) levels of 1,4-BQ-Alb. It included effects for gender, BMI (log-transformed), automobile refuelling, smoking status, as well as consumption of fruits and the artificial sweetener, aspartame. For example, the levels of 1,4-BQ-Alb were higher for smokers ($e^{0.142}$ or 1.15 times larger) than for non-smokers after adjustment for the other explanatory variables. Additionally, except for the interaction of gender with BMI, no significant interactions were found ($p > 0.10$). This multiple regression model explained about 21% of total variation of baseline levels of 1,4-BQ-Alb across individuals. Other potentially important covariates from univariate analyses, daily coffee and tea intake, racial group, alcohol consumption, phenylalanine intake and medicine use were not significant ($p > 0.10$) after adjusting for the effects shown in Table III. Caloric intake was neither a significant predictor (estimated regression coefficient $\pm$ SE $= 1.01 \times 10^{-6} \pm 3.50 \times 10^{-5}$, $p = 0.98$) nor a potential confounder in the multiple regression model (data not shown).

Levels of 1,4-BQ-Alb from the current study are compared in Figure 2 with those of 51 adult control workers from a previous study conducted in China (Rappaport et al. 2002). Note that the 1,4-BQ-Alb levels among unexposed Chinese workers were higher in males than females ($p < 0.001$, Wilcoxon rank-sum test) in contrast to results from our US subjects where females had higher levels than males ($p < 0.001$, Wilcoxon rank-sum test). We considered the possibility that this difference might reflect smoking patterns in China where most males smoked and most females did not smoke. However, among non-smoking subjects from the Chinese study, males still had higher 1,4-BQ-Alb levels (median = 1320 pmol g^{-1}) than females (median = 874 pmol g^{-1}, $p = 0.007$, Wilcoxon rank-sum test).

Variation of adduct levels within and between subjects

Second blood samples were used from 11 males and 22 females to estimate the variance components in the measurements of 1,4-BQ-Alb. Profile plots for subjects with second blood samples are shown in Figure 3. The median time interval between repeated measurements of 1,4-BQ-Alb was 98 days (range 42–123 days), which is much longer than the reported 13.5-day half-time of 1,4-BQ-Alb in human serum

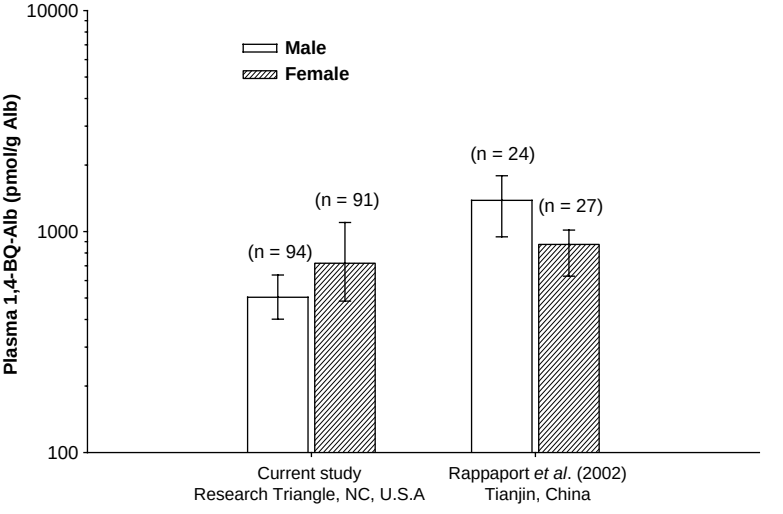

Figure 2. Comparison of plasma 1,4-BQ-Alb levels between non-occupationally exposed individuals in the current study and those in Rappaport et al. (2002). Error bars represent standard errors.

(Rappaport et al. 2002). Thus, it is reasonable to expect that the estimated variance components were not influenced by the autocorrelation of repeated blood samples in this study. Under Model (1), the estimated between-person variance component ($\hat{\sigma}^2_B = 0.148$) was approximately the same as the within-person variance component ($\hat{\sigma}^2_W = 0.156$) for the 33 subjects with two blood samples (intraclass correlation coefficient $\hat{\rho} = 0.490$). Since some of the within-subject variance is derived from the assay error, given by a coefficient of variation of 0.20, which is equivalent to an assay variance component (in log scale) of 0.039, the estimated intraclass correlation

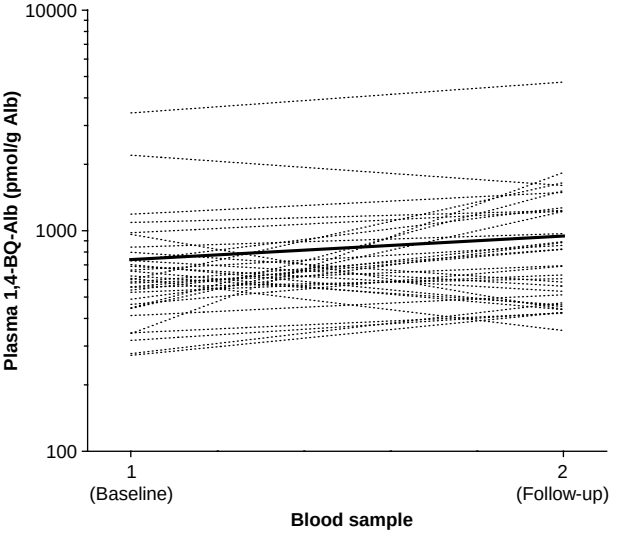

Figure 3. Profile plots of levels of 1,4-BQ-Alb ($n = 33$ subjects). Two blood samples were collected, on average, about 3 months apart. Each dashed-line represents one subject. The solid line represents the mean trend.

$\hat{\rho} = \dfrac{0.148}{0.148 + (0.156 - 0.039)} = 0.558$ becomes marginally larger after adjustment for the assay variance. In any case, these values of $\hat{\rho}$ indicate that there is substantial variability in levels of 1,4-BQ-Alb within subjects over time. The estimated between- and within-person variance components were about the same when Model (1) was applied to all subjects with and without repeats ($n = 221$ measurements), i.e. $\hat{\sigma}^2_B = 0.153$ and $\hat{\sigma}^2_W = 0.155$.

Discussion

The present findings support the hypothesis that background exposures to benzene, phenol and hydroquinone ultimately produce 1,4-BQ, a toxicant that has been implicated in haematoxicity and leukaemia from benzene exposure (McDonald et al. 1993, Smith 1996). The following significant predictors of 1,4-BQ-Alb were identified in the present analyses: gender, BMI, the gender–BMI interaction, cigarette smoking, automobile refuelling, and consumption of the artificial sweetener, aspartame, and fruit.

The apparent associations between 1,4-BQ-Alb and automobile refuelling (more than five times/month) and smoking are likely due to inhalation of benzene. Airborne exposure to benzene has been documented during self-service automobile refuelling (Egeghy et al. 2000) and tobacco smoke is a major source of benzene exposure in the general population (IARC 1989, Wallace 1996). The effect of smoking upon 1,4-BQ-Alb levels could also be influenced by co-exposure to hydroquinone in cigarette smoke (Ong et al. 1994, Deisinger et al. 1996).

The strongest effect identified in our analyses was BMI ($p = 0.001$), where the levels of 1,4-BQ-Alb increased with decreasing BMI, particularly for females (due to the gender–BMI interaction) and among African-Americans, who tended to have higher BMIs than Caucasians ($p = 0.002$). The increased adduct levels observed among subjects with lower BMIs is consistent with evidence that lung cancer risks and DNA adduct levels in peripheral blood lymphocytes were inversely related to BMI (Godschalk et al. 2002). Since metabolic activity tends to be greater in lean subjects than in overweight subjects (Ravussin et al. 1988), this finding could be related to more efficient metabolism of benzene and phenol in subjects with low BMIs. Alternatively, benzene and phenol are lipophilic substances that can be sequestered in adipose tissue and therefore are unavailable for metabolism.

The present finding that females had higher adduct levels than males runs counter to previous results from unexposed workers in Tianjin, China (Rappaport et al. 2002), where males had higher adduct levels (Figure 2). Since the incidence of AML among US males is higher than that among females (Bergsagel et al. 1999), the present results tend to weaken the hypothesis that 1,4-BQ is a risk factor for AML. In any case, the gender differences in adduct levels between the current study and the previous Chinese study might be related to the racial background or to dietary, lifestyle and environmental differences between the populations. Differences in the distributions of CYP2E1 and NQO1 gene alleles have been reported between Asian and Caucasian populations (Hildesheim et al. 1997, Gaedigk et al. 1998, Zhang et al. 2003). Also, females from the current investigation were more frequent users of medications, a factor that was associated with higher levels of 1,4-BQ-Alb in univariate analyses (Table II). Results of adduct determinations in larger samples of persons will need to be.

We also detected rather weak associations between 1,4-BQ-Alb levels and consumption of fruit ($p = 0.074$) and the artificial sweetener, aspartame ($p = 0.059$). The apparent association with fruit consumption is consistent with hypotheses proposed by Deisinger et al. (1996) and McDonald et al. (2001) that arbutin-rich fruits would be important sources of hydroquinone. Metabolism of aspartame would presumably release phenylalanine (Butchko et al. 2002) and eventually phenol (McDonald et al. 2001). (Acceptable daily intake of aspartame has been established at 50 mg kg^{-1} day^{-1} in the USA (FDA 1984)). However, consumption of conventional foods rich in phenylalanine was not a significant predictor of 1,4-BQ-Alb, after adjustment for other variables in our final model (Table III).

Because second blood samples from a subset of study participants were obtained, a linear mixed model was used to estimate the between- and within-person variance components of (logged) levels of 1,4-BQ-Alb in the general population. Large estimates of both between- and within-person variance components in these adduct levels were observed. This indicates that variability in levels of 1,4-BQ-Alb in the population was due to differences in fixed-effect predictors (such as those shown in Table III) among subjects as well as to random changes in dietary and environmental sources of 1,4-BQ-Alb that occurred within subjects over time.

The estimated parameters from the multiple regression model can be used to predict levels of benzene exposure that would produce equivalent adduct levels. Using the estimated parameters given in Table III, subjects in the population had expected levels of 1,4-BQ-Alb between 394 pmol g^{-1} Alb (i.e. $\exp(8.860 - 0.800 \times \ln(25) - 2.597 + 0.711 \times \ln(25))$, representing non-smoking males with a BMI of 25 kg m^{-2}, who refuelled their automobiles no more than five times/month and had low intake of aspartame (≤ 1 time/month) and fruits (less than or equal to three times/week)), and 971 pmol g^{-1} Alb (i.e. $\exp(8.860 - 0.\ 800 \times \ln(25) + 0.142 + 0.176 + 0.141 + 0.134)$), representing smoking females with a BMI of 25 kg m^{-2}, who refuelled their automobiles more than five times/month, and had high intake of aspartame and fruits. It was previously estimated that workers exposed to non-saturating levels of benzene produced 569 pmol 1,4-BQ-Alb g^{-1} Alb per ppm benzene per workday (Rappaport et al. 2002). Thus, if all the observed 1,4-BQ-Alb in the current study had been produced by occupational exposure to benzene, the equivalent air concentrations would have been between 1.08 and 3.64 ppm (3.45–11.6 mg m^{-3}) benzene.

In conclusion, gender, physiological, environmental, lifestyle and dietary factors that affected levels of 1,4-BQ-Alb adducts in plasma have been identified. Although it is recognized that data generated from food-frequency questionnaires are imprecise and susceptible to recall bias (Willett 1998), the present authors are encouraged that most of the associations found are mechanistically reasonable. Since it has been suggested that 1,4-BQ is responsible for much of the haematotoxicity and leukaemogenicity of occupational benzene exposure, the surprisingly high levels of 1,4-BQ-Alb observed here and in previous studies raise the question of whether background incidence of diseases of the haematopoietic system, including AML, may be related to 1,4-BQ in the general population. The analysis of the within- and between-person variance components of levels of 1,4-BQ-Alb suggests that background levels of 1,4-BQ-Alb vary greatly among individuals, but also vary significantly within the same persons over time.

Acknowledgements

Blood was collected with assistance from the General Clinical Research Center, University of North Carolina School of Medicine. The authors are grateful to Christina Martinez, who performed the assays for albumin adducts. They also appreciate the contributions of Qingshan Qu, Roy Shore, Beverley Cohen, Songnian Yin and Guilan Li, who provided data from an earlier investigation of Chinese control workers. The authors received technical assistance regarding sources of dietary phenols from the Nutrition Core of the UNC Center for Environmental Health and Susceptibility. They are also indebted to two anonymous reviewers who noted that several aspects of the findings were not addressed in the original submission. The study was supported in part by a Population Sciences Research Award from the UNC Lineberger Comprehensive Cancer Center, by Grants Nos P42ES05948 and P30ES10126 from the National Institute for Environmental Health Sciences, and by Grant No. R01CA69463 from the National Cancer Institute.

References

Aspartame Information Center. 2005 (available at: http://www.aspartame.org) (accessed on 28 August 2005).

Bergsagel DE, Wong O, Bergsagel PL, Alexanian R, Anderson K, Kyle RA, Raabe GK. 1999. Benzene and multiple myeloma: appraisal of the scientific evidence. Blood 94:1174–1182.

Butchko HH, Stargel WW, Comer CP, Mayhew DA, Benninger C, Blackburn GL, de Sonneville LM, Geha RS, Hertelendy Z, Koestner A, et al. 2002. Aspartame: review of safety. Regulations in Toxicology and Pharmacology 35:S1–S93.

Deisinger PJ, Hill TS, English JC. 1996. Human exposure to naturally occurring hydroquinone. Journal of Toxicology and Environmental Health 47:31–46.

Egeghy PP, Tornero-Velez R, Rappaport SM. 2000. Environmental and biological monitoring of benzene during self-service automobile refueling. Environmental Health Perspectives 108:1195–1202.

FDA. 1984. Food additives permitted for direct addition to food for human consumption: aspartame. Food and Drug Administration. Federal Register 49:6672.

Gaedigk A, Tyndale RF, Jurima-Romet M, Sellers EM, Grant DM, Leeder JS. 1998. NAD(P)H: quinone oxidoreductase: polymorphisms and allele frequencies in Caucasian, Chinese and Canadian Native Indian and Inuit populations. Pharmacogenetics 8:305–313.

Glass DC, Gray CN, Jolley DJ, Gibbons C, Sim MR, Fritschi L, Adams GG, Bisby JA, Manuell R. 2003. Leukemia risk associated with low-level benzene exposure. Epidemiology 14:569–577.

Godschalk RW, Feldker DE, Borm PJ, Wouters EF, Van Schooten FJ. 2002. Body mass index modulates aromatic DNA adduct levels and their persistence in smokers. Cancer Epidemiology and Biomarkers Prevention 11:790–793.

Hayes RB, Yin S, Rothman N, Dosemeci M, Li G, Travis LT, Smith MT, Linet MS. 2000. Benzene and lymphohematopoietic malignancies in China. Journal of Toxicology and Environmental Health A 61:419–432.

Hildesheim A, Anderson LM, Chen CJ, Cheng YJ, Brinton LA, Daly AK, Reed CD, Chen IH, Caporaso NE, Hsu MM, et al. 1997. CYP2E1 genetic polymorphisms and risk of nasopharyngeal carcinoma in Taiwan. Journal of the National Cancer Institute 89:1207–1212.

Hu FB, Rimm E, Smith-Warner SA, Feskanich D, Stampfer MJ, Ascherio A, Sampson L, Willett WC. 1999. Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. American Journal of Clinical Nutrition 69:243–249.

IARC. 1989. Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Benzene, Vol. 45. Lyon: International Agency for Research on Cancer.

Lan Q, Zhang L, Li G, Vermeulen R, Weinberg RS, Dosemeci M, Rappaport SM, Shen M, Alter BP, Wu Y, et al. 2004. Hematotoxicity in workers exposed to low levels of benzene. Science 306:1774–1776.

Lindstrom AB, Yeowell-O'Connell K, Waidyanatha S, McDonald TA, Golding BT, Rappaport SM. 1998. Formation of hemoglobin and albumin adducts of benzene oxide in mouse, rat, and human blood. Chemical Research in Toxicology 11:302–310.

McDonald TA, Holland NT, Skibola C, Duramad P, Smith MT. 2001. Hypothesis: phenol and hydroquinone derived mainly from diet and gastrointestinal flora activity are causal factors in leukemia. Leukemia 15:10–20.

McDonald TA, Waidyanatha S, Rappaport SM. 1993. Measurement of adducts of benzoquinone with hemoglobin and albumin. Carcinogenesis 14:1927–1932.

Ong CN, Lee BL, Shi CY, Ong HY, Lee HP. 1994. Elevated levels of benzene-related compounds in the urine of cigarette smokers. International Journal of Cancer 59:177–180.

OSHA. 1987. Occupational exposure to benzene. Federal Register 52:34460–34579.

Peters JM, Preston-Martin S, London SJ, Bowman JD, Buckley JD, Thomas DC. 1994. Processed meats and risk of childhood leukemia. Cancer Causes and Control 5:195–202.

Rappaport SM, Waidyanatha S, Qu Q, Shore R, Jin X, Cohen B, Chen LC, Melikian AA, Li G, Yin S, et al. 2002. Albumin adducts of benzene oxide and 1,4-benzoquinone as measures of human benzene metabolism. Cancer Research 62:1330–1337.

Ravussin E, Lillioja S, Knowler WC, Christin L, Freymond D, Abbott WG, Boyce V, Howard BV, Bogardus C. 1988. Reduced rate of energy expenditure as a risk factor for body-weight gain. New England Journal of Medicine 318:467–472.

Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. 1992. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. American Journal of Epidemiology 135:1114–1126; discussion, 1127–1136.

Salvini S, Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B, Willett WC. 1989. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. International Journal of Epidemiol 18:858–867.

Savitz DA, Andrews KW. 1997. Review of epidemiologic evidence on benzene and lymphatic and hematopoietic cancers. American Journal of Industrial Medicine 31:287–295.

Smith EA, Macfarlane GT. 1996. Enumeration of human colonic bacteria producing phenolic and indolic compounds: effects of pH, carbohydrate availability and retention time on dissimilatory aromatic amino acid metabolism. Journal of Applied Bacteriology 81:288–302.

Smith MT. 1999. Benzene, NQO1, and genetic susceptibility to cancer. Proceedings of the National Academy of Sciences USA 96:7624–7626.

Smith MT. 1996. The mechanism of benzene-induced leukemia: a hypothesis and speculations on the causes of leukemia. Environmental Health Perspectives 104(Suppl. 6):1219–1225.

Snyder R. 2002. Benzene and leukemia. Critical Reviews in Toxicology 32:155–210.

Snyder R. 2000. Overview of the toxicology of benzene. Journal of Toxicology and Environmental Health A 61:339–346.

Waidyanatha S, Yeowell-O'Connell K, Rappaport SM. 1998. A new assay for albumin and hemoglobin adducts of 1,2- and 1,4-benzoquinones. Chemico-Biological Interactions 115:117–139.

Wallace L. 1996. Environmental exposure to benzene: an update. Environmental Health Perspectives 104(Suppl. 6):1129–1136.

Whysner J, Reddy MV, Ross PM, Mohan M, Lax EA. 2004. Genotoxicity of benzene and its metabolites. Mutation Research 566:99–130.

Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. 1985. Reproducibility and validity of a semiquantitative food frequency questionnaire. American Journal of Epidemiology 122:51–65.

Willett WC. 1998. Nutritional epidemiology. New York: Oxford University Press.

Yeowell-O'Connell K, Rothman N, Waidyanatha S, Smith MT, Hayes RB, Li G, Bechtold WE, Dosemeci M, Zhang L, Yin S, et al. 2001. Protein adducts of 1,4-benzoquinone and benzene oxide among smokers and nonsmokers exposed to benzene in China. Cancer and Epidemiology Biomarkers Prevention 10:831–838.

Zhang J, Schulz WA, Li Y, Wang R, Zotz R, Wen D, Siegel D, Ross D, Gabbert HE, Sarbia M. 2003. Association of NAD(P)H: quinone oxidoreductase 1 (NQO1) C609T polymorphism with esophageal squamous cell carcinoma in a German Caucasian and a northern Chinese population. Carcinogenesis 24:905–909.