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Protein adducts as biomarkers of human benzene metabolism

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Abstract

We used cysteinyl adducts of serum albumin (Alb) to investigate the production of two reactive benzene metabolites, namely, benzene oxide (BO) and 1,4-benzoquinone (1,4-BQ) in workers exposed to benzene. Adducts were measured in 160 benzene-exposed workers who did not use respiratory protection (based upon individual geometric mean benzene exposure levels: median = 5.27 ppm, interquartile range = 2.14–13.4 ppm, range = 0.074–328 ppm) and 101 local controls, from populations in Shanghai and Tianjin, China. After isolation of Alb, these adducts (designated as BO-Alb and 1,4-BQ-Alb) were cleaved from the protein with methanesulfonic acid and trifluoroacetic anhydride and measured by gas chromatography-mass spectrometry. Although BO-Alb and 1,4-BQ-Alb were measured in all subjects, levels of both adducts were 2.4-fold greater (median value) in exposed subjects than in controls (interquartile-fold range = 1.63–4.05 for BO-Alb and 1.64–3.69 for 1,4-BQ-Alb). Log–log plots of the individual adduct levels versus exposure were quasi-linear with straight-line slopes of about 0.3 for both BO-Alb and 1,4-BQ-Alb. Since these log-space slopes were significantly less than one, we infer that adduct production was nonlinear, i.e., less-than proportional to benzene exposure, over the indicated range. This behavior points to saturation of CYP2E1 as a critical metabolic consequence of high exposure to benzene in humans. Thus, the biologically effective dose of BO and 1,4-BQ should be proportionally greater in persons exposed to low rather than high levels of benzene.

Keywords: Benzene; Protein adducts; Albumin; Benzene oxide; Benzoquinone; Nonlinear kinetics

1. Introduction

Benzene is carcinogenic in both experimental animals and humans, presumably due to toxic effects in the bone marrow arising from its metabolism [1,2]. Although benzene is the simplest aromatic compound,

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its metabolism is surprisingly complex. Following CYP2E1 oxidation of benzene to benzene oxide (BO) [3,4], a series of enzymatic and nonenzymatic steps lead to many other metabolites, notably, phenol, catechol, hydroquinone, and the ring-opened muconaldehydes (ultimately transformed to *t*,*t*-muconic acid). Portions of catechol and hydroquinone are oxidized to 1,2- and 1,4-benzoquinone, respectively, which are electrophilic and capable of reacting with DNA and other critical macromolecules, as are BO and the muconaldehydes. The toxicity of benzene is thought to involve one or more of these electrophilic metabolites (most notably 1,4-BQ) and/or reactive oxygen species, produced by redox cycling of catechol, hydroquinone and the respective benzoquinones [1,2].

Human metabolism of benzene has largely been inferred from in vitro experiments [5–10], by measurements of urinary metabolites in persons following occupational exposure [11–16], and by physiologically based toxicokinetic models [17–20]. While undoubtedly useful, these methods provide relatively little information about the biologically effective doses of the electrophilic benzene metabolites, whose residence times are very short in vivo (a few minutes at most). This motivated us to apply stable adducts of benzene's electrophilic intermediates, i.e., BO and 1,4-benzoquinone (1,4-BQ) with Hb and/or albumin (Alb), in the blood of benzene-exposed workers as measures of the biologically effective dose [21–24].

Our studies of protein adducts relied upon populations of workers, exposed to a wide range of airborne benzene levels, and concurrent controls in Shanghai [21–23] and Tianjin, China [24]. We previously reported exposure–adduct relationships in these two populations, focusing primarily upon the Alb adducts, which were more abundant than those of Hb in our samples. In both cases, significant departures from linear kinetics were observed, suggesting saturation of benzene metabolism [23,24]. In the current investigation we combine results of the Alb adducts from the two studies to further scrutinize 160 subjects who were exposed to benzene from less than 1 ppm to greater than 300 ppm. Since significant and varying background levels of BO-Alb and 1,4-BQ-Alb were observed among 101 control subjects in these studies, we express adduct levels of exposed workers as fold increases above concurrent control values prior to examining the exposure–adduct relationships. Results indicate that subjects were exposed over the full range of kinetic processes from linear (first-order) metabolism at low exposures to essentially saturated metabolism at high exposures.

2. Measurements of benzene exposure and adducts

The recruitment of subjects and analytical methods have been described in detail elsewhere [21–26]. Briefly, the first study was sponsored by the National Cancer Institute (hereafter the 'NCI study'). From the NCI study, adduct measurements were available from 43 exposed workers and 50 controls of both sexes from Shanghai, China. Of the exposed workers, 16 wore respirators and were excluded from the current analyses. Each exposed worker had 5 or 6 personal full-shift air measurements of benzene during the week prior to blood collection (using organic vapor monitors and gas chromatography). The geometric mean (GM) of these individual personal measurements was used as the measure of individual benzene exposure. The median level of benzene exposure (individual GM value) among the 27 workers from the NCI study (who did not use respirators) was 17.2 ppm (interquartile range: 13.5–92.9 ppm, range: 1.65–328 ppm). The second study was sponsored by the Health Effects Institute (hereafter the 'HEI study'). From the HEI study, adduct measurements were available from 133 exposed workers and 51 controls of both sexes from Tianjin, China. Each exposed subject had between 1 and 6 personal full-shift measurements (111 subjects had 4 measurements) of benzene exposure, obtained weekly during the month prior to blood collection (also measured with organic vapor monitors and gas chromatography). Again, the individual GM was used as the measure of benzene exposure. The median level of benzene exposure among the 133 exposed workers from the HEI study was 3.70 ppm (interquartile range: 2.03–10.0 ppm, range: 0.074–46.6 ppm). Combining results from the NCI and HEI studies, the median level of benzene exposure among the 160 exposed workers was 5.27 ppm (interquartile range: 2.14–13.4 ppm, range: 0.074–328 ppm).

Adducts of serum Alb were measured with an assay, developed in our laboratory, which selectively cleaved

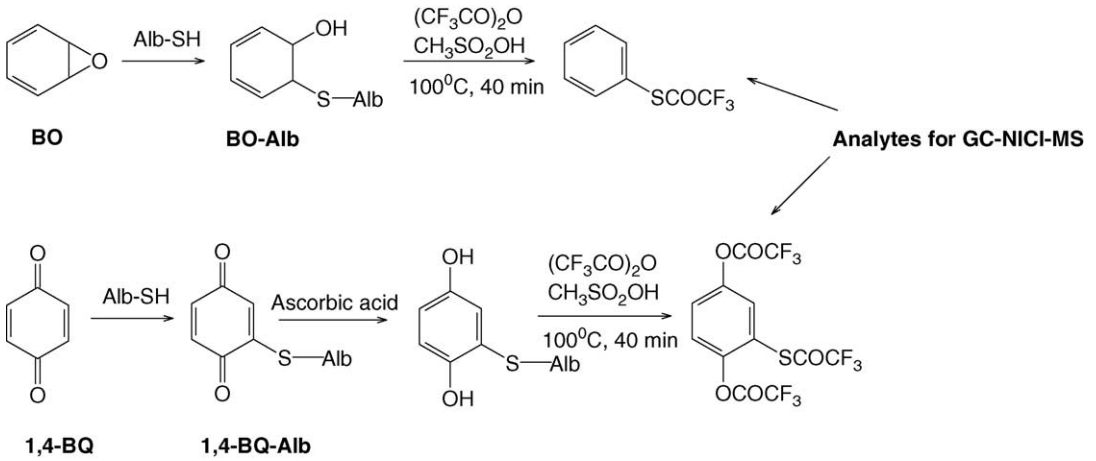

Fig. 1. Scheme for analysis of albumin adducts of benzene oxide (BO-Alb) and 1,4-benzoquinone (1,4-BQ-Alb). (GC–NICI–MS refers to gas chromatography–mass spectrometry with negative ion chemical ionization).

cysteinyl adducts from the protein and converted BO-Alb and 1,4-BQ-Alb into volatile derivatives, suitable for gas chromatography-mass spectrometry. As shown in Fig. 1, these analytes were generated by reacting 3–5 mg of dried Alb with trifluoroacetic anhydride and methanesulfonic acid. All adduct levels were based upon a single blood specimen from each subject. The precision of the assay was estimated from coefficients of variation of 0.30 for BO-Alb and 0.20 for 1,4-BQ-Alb [24].

Table 1 summarizes the adduct levels for exposed and control subjects in the two studies. Among control subjects, levels of BO-Alb were significantly greater in the HEI study (median = 174 pmol/g Alb) than in the NCI study (median = 115 pmol/g Alb) while levels of 1,4-BQ-Alb were significantly greater in the NCI study (median = 2270 pmol/g Alb) than in the HEI study (median = 994 pmol/g Alb) ($p < 0.0001$ in both cases by the Wilcoxon test). While the reasons for these differences have not been fully elucidated, the much larger control values of 1,4-BQ-Alb in the NCI study can be largely attributed to collection of blood in EDTA rather than in citrate, as for the HEI study (based upon unpublished experiments in our laboratory). Other differences in background adducts were probably not greatly influenced by environmental exposures to benzene, but rather to smoking and unexplained dietary and endogenous factors that contributed phenol, hydroquinone, and other precursor molecules in vivo [21,22,24,27].

To adjust for differences in background adduct levels across studies, we express each exposed subject's adduct level as a fold increase above the control value. That is, the Relative Adduct Level (RAL) of the *i*th exposed worker in the *h*th study is given by $RAL_{hi} = A_{hi}/C_h$, where A_{hi} is the observed adduct level for that worker and C_h is the estimated GM adduct level of control workers in that study. In our samples, $C_h = 115$ and 169 pmol/g Alb for BO-Alb, and $C_h = 2490$ and 1030 pmol/g Alb for 1,4-BQ-Alb in studies 1 (NCI) and 2 (HEI), respectively. The last three columns of Table 1 show the median RAL_{hi} values, along with the interquartile ranges and ranges for the NCI and HEI studies. Interestingly, while the median level of BO-Alb was significantly greater for the NCI study (median = 3.82-fold) than the HEI study (median = 2.19-fold) ($p = 0.0003$, Wilcoxon test) that for 1,4-BQ-Alb was not (median = 2.34-fold for the NCI study versus 2.42 for the HEI study) ($p = 0.933$, Wilcoxon test). With data combined from both the NCI and HEI studies, the median value of RAL_{hi} was 2.4-fold for both adducts (interquartile range = 1.63–4.05 for BO-Alb and 1.64–3.69 for 1,4-BQ-Alb).

3. Exposure–adduct relationships

Scatter plots showing RAL_{hi} values for BO-Alb and BQ-Alb versus benzene exposure are given in Fig. 2

Table 1
Levels of albumin adducts of benzene oxide (BO-Alb) and 1,4-benzoquinone (1,4-BQ-Alb) in the serum of workers from the NCI and HEI studies

Study	Adduct	Adduct level (pmol/g Alb)								Relative adduct level (fold increase above controls)		
		Control workers				Exposed workers				Exposed workers		
		n	Median	IQR[a]	Range	*n*	Median	IQR[a]	Range	Median	IQR[a]	Range
NCI	BO-Alb	50	115	91.9–158	44.3–250	27	440	323–757	160–2240	3.82	2.80–6.57	1.39–19.5
NCI	1,4-BQ-Alb	50	2270	1630–3610	960–9410	27	5830	4100–8320	2410–16000	2.34	1.64–3.34	0.969–6.41
HEI	BO-Alb	51	174	124–204	82.0–544	133	371	266–605	100–7465	2.19	1.57–3.57	0.591–44.1
HEI	1,4-BQ-Alb	51	994	709–1540	427–2570	133	2490	1590–4000	265–10400	2.42	1.55–3.89	0.258–10.1

[a] Interquartile range.

with different symbols representing the NCI and HEI subjects. The data appear to be consistent across studies, as indicated by the superposition of observations in the region of overlapping benzene exposure, between about 2 and 30 ppm. The scatter plots also indicate that the log–log relationships between relative adduct levels and benzene exposure are reasonably linear over the indicated range of exposures.

After combining data from both studies, the (natural) logged RAL_{hi} values for the two adducts were regressed upon the logged benzene exposure levels as shown in Fig. 3A. Since the outcome variable $RAL_{hi} = A_{hi}/C_h$ is the ratio of the adduct level of the *i*th subject from group *h* to the GM adduct level for group *h*, note that these straight-line models were regressing $[\ln(A_{hi}) - \ln(C_h)]$, representing the logged individual adduct level minus the mean of the logged control adduct levels, on ln(*Benzene Exposure*). For both adducts, the fits to the straight-line model were reason-

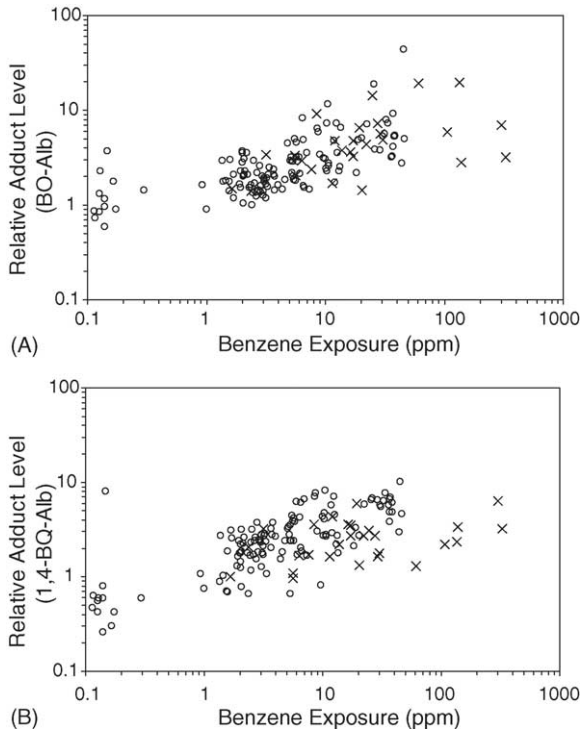

Fig. 2. Scatter plots of relative adduct levels (RAL, fold increase above control values) vs. benzene exposure for 160 workers from the NCI (×) and HEI (open symbol) studies. (A) Benzene oxide-albumin adducts; (B) 1,4-benzoquinone–albumin adducts.

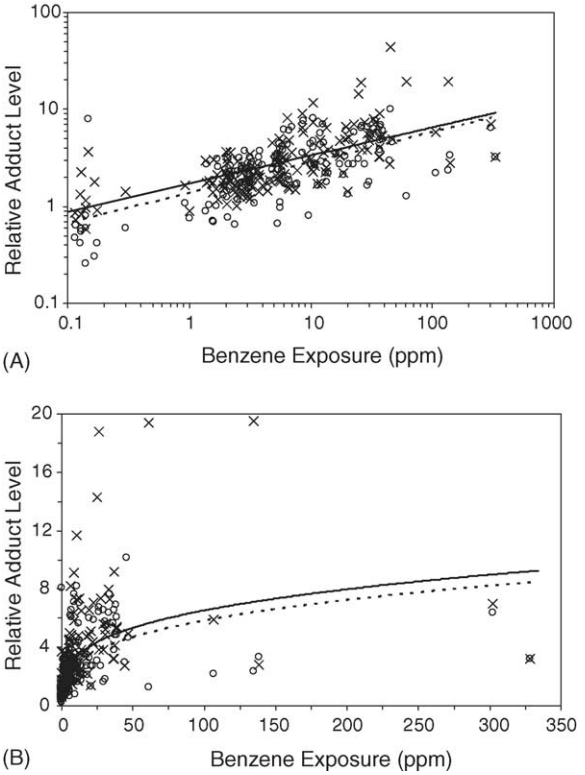

Fig. 3. Relative adduct levels (RAL, fold increase above control values) vs. benzene exposure for benzene oxide-albumin adducts (×) and 1,4-benzoquinone–albumin adducts (open symbol) from 160 workers combined from the NCI and HEI studies. Lines represent the predictions from least-squares regression of the natural logarithm of RAL on the natural logarithm of benzene exposure. (A) Log-space relationships; (B) natural-space relationships.

able with slopes of about 0.3 (BO-Alb: slope = 0.289, S.E. = 0.024, $R^2 = 0.480$; 1,4-BQ-Alb: slope = 0.312, S.E. = 0.026, $R^2 = 0.485$). Since these log–space slopes were significantly less than one (upper 95% confidence limits were 0.337 and 0.362 for BO-Alb and 1,4-BQ-Alb, respectively), we infer that the corresponding natural-space relationships were substantially nonlinear with concave-downwards shapes. This is illustrated in Fig. 3B, which shows the RAL_{hi} values plotted versus benzene exposure in natural-space along with the predicted values from the log-space regressions. Despite the large variability, apparent for both adducts at a given benzene exposure, the exposure–adduct relationships reached plateaus at higher benzene levels, suggesting that CYP2E1 was substantially saturated at about 50 ppm of benzene. Although the initial point of departure from linear kinetics cannot be inferred from Fig. 3B, we previously estimated this point to be about 1 ppm, based upon the HEI data, which provide most of the low-exposed subjects in the current analysis [24].

4. Implications for health risk

As noted in the introduction, protein adducts offer the opportunity to study the disposition of reactive electrophiles that cannot readily be measured in vivo. In this application, we used cysteinyl adducts of serum Alb to evaluate the production of two transient benzene metabolites, i.e., BO and 1,4-BQ over a wide range of benzene exposures (0.07–328 ppm). We previously reported that BO-Alb was chemically stable in vivo, being eliminated with a presumed half time of 21 days in humans, and that 1,4-BQ-Alb was moderately stable, with an estimated elimination half time of 13.5 days in humans [24]. Thus, these adducts reflect the integration of exposure over 3 or 4 weeks prior to blood collection and are relatively insensitive to day-to-day variability in exposure that can obscure exposure–biomarker relationships [28].

The relevance of BO-Alb and 1,4-BQ-Alb to human health risk should be considered separately. Since BO is formed during the initial CYP2E1 oxidation of benzene, BO-Alb represents a global measure of benzene metabolism and, thus, should be unequivocally relevant to health risk, insofar as metabolism appears to be a necessary condition for benzene to cause leukemia and toxic effects of the bone marrow. Benzoquinone, on the other hand, is formed following nonenzymatic rearrangement of BO to phenol, CYP2E1 oxidation of phenol (to hydroquinone), and oxidation of hydroquinone to 1,4-BQ, either spontaneously or via peroxidases [1,2]. Thus, formation of 1,4-BQ is subject to several factors involving the dispositions of BO, phenol and hydroquinone (e.g., reactions with glutathione and other nucleophiles, oxidizing and reducing enzymes, conjugation with sulfate, etc.) as well as the competitive inhibition of CYP2E1 by BO and phenol. As such, 1,4-BQ-Alb is less useful than BO-Alb as a global measure of benzene metabolism but is arguably more relevant to the health risk of benzene, insofar as 1,4-BQ is widely thought to be an important toxic metabo-

lite of benzene [1,2]. But in either case, the concave downwards production of BO-Alb and 1,4-BQ-Alb, observed at increasing exposure to benzene, point to the initial CYP2E1 oxidation as a key factor in modulating human health risk of benzene.

Finally, it is worth mentioning that the concave downwards shapes of the exposure–adduct relationships shown in Fig. 3B, suggest that metabolism per unit of benzene exposure should be much more effective at low air levels than at high air levels of benzene [24]. This has two important implications for risk assessment. First, in occupational studies where full-shift benzene levels can be very high, one would anticipate that transient days of exposure greater than about 50 ppm would have diminished impact upon the health risk, since benzene metabolism is substantially saturated in this region (Fig. 3B). This would lead to a concave downwards shape in the exposure–leukemia incidence for benzene, as reported by Hayes et al. [29], and would reduce the impact of high transient (peak) exposures to benzene upon leukemia risk, as observed by Glass et al. [30]. Second, our results indicate that environmental exposure to benzene, which occurs at air levels less than 1 ppm [31], would produce the maximum possible mass of metabolites per unit of benzene exposure. Since essentially every human is exposed to low levels of benzene from use of hydrocarbon fuels and tobacco [32], the potential for benzene to induce leukemia following long-term environmental exposure should not be dismissed out of hand.

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References

[1] R. Snyder, Benzene and leukemia, Crit. Rev. Toxicol. 32 (2002) 155–210.
[2] R. Snyder, Overview of the toxicology of benzene, J. Toxicol. Environ. Health A 61 (2000) 339–346.
[3] A.B. Lindstrom, K. Yeowell-O'Connell, S. Waidyanatha, B.T. Golding, R. Tornero-Velez, S.M. Rappaport, Measurement of benzene oxide in the blood of rats following administration of benzene, Carcinogenesis 18 (1997) 1637–1641.
[4] M.R. Lovern, M.J. Turner, M. Myer, G.L. Kedderis, W.E. Bechtold, P.M. Schlosser, Identification of benzene oxide as a product of benzene metabolism by mouse, rat, and human liver microsomes, Carcinogenesis 18 (1997) 1695–1700.
[5] D.A. Eastmond, D.S. Rupa, L.S. Hasegawa, Detection of hyperdiploidy and chromosome breakage in interphase human lymphocytes following exposure to the benzene metabolite hydroquinone using multicolor fluorescence in situ hybridization with DNA probes, Mutat. Res. 322 (1994) 9–20.
[6] C.E. Frantz, H. Chen, D.A. Eastmond, Inhibition of human topoisomerase II in vitro by bioactive benzene metabolites, Environ. Health Perspect 104 (Suppl. 6) (1996) 1319–1323.
[7] I. Gut, V. Nedelcheva, P. Soucek, P. Stopka, B. Tichavska, Cytochromes P450 in benzene metabolism and involvement of their metabolites and reactive oxygen species in toxicity, Environ. Health Perspect 104 (Suppl. 6) (1996) 1211–1218.
[8] R.D. Irons, W.S. Stillman, Impact of benzene metabolites on differentiation of bone marrow progenitor cells, Environ. Health Perspect 104 (Suppl. 6) (1996) 1247–1250.
[9] P. Kolachana, V.V. Subrahmanyam, K.B. Meyer, L. Zhang, M. Smith, Benzene and its phenolic metabolites produce oxidative DNA damage in HL60 cells in vitro and in the bone marrow in vivo, Cancer Res. 53 (1993) 1023–1026.
[10] J.W. Yager, D.A. Eastmond, M.L. Robertson, W.M. Paradisin, M.T. Smith, Characterization of micronuclei induced in human lymphocytes by benzene metabolites, Cancer Res. 50 (1990) 393–399.
[11] N.J. van Sittert, P.J. Boogaard, G.D.J. Beulink, Application of the urinary *S*-phenylmercapturic acid test as a biomarker for low levels of exposure to benzene in industry, Br. J. Ind. Med. 50 (1993) 460–469.
[12] N. Rothman, W.E. Bechtold, S.N. Yin, M. Dosemeci, G.L. Li, Y.Z. Wang, W.C. Griffith, M.T. Smith, R.B. Hayes, Urinary excretion of phenol, catechol, hydroquinone, and muconic acid by workers occupationally exposed to benzene, Occup. Environ. Med. 55 (1998) 705–711.
[13] A.A. Melikian, R. O'Connor, A.K. Prahalad, P. Hu, H. Li, M. Kagan, S. Thompson, Determination of the urinary benzene metabolites *S*-phenylmercapturic acid and trans,trans-muconic acid by liquid chromatography-tandem mass spectrometry, Carcinogenesis 20 (1999) 719–726.
[14] S. Waidyanatha, N. Rothman, S. Fustinoni, M.T. Smith, R.B. Hayes, W. Bechtold, M. Dosemeci, G. Li, S. Yin, S.M. Rappaport, Urinary benzene as a biomarker of exposure among occupationally exposed and unexposed subjects, Carcinogenesis 22 (2001) 279–286.

[15] B.L. Lee, A.L. New, P.W. Kok, H.Y. Ong, C.Y. Shi, C.N. Ong, Urinary trans,trans-muconic acid determined by liquid chromatography: application in biological monitoring of benzene exposure, Clin. Chem. 39 (1993) 1788–1792.
[16] S. Waidyanatha, N. Rothman, G. Li, M.T. Smith, S. Yin, S.M. Rappaport, Rapid determination of six urinary benzene metabolites in occupationally exposed and unexposed subjects, Anal. Biochem. 327 (2004) 184–199.
[17] F.Y. Bois, T.J. Woodruff, R.C. Spear, Comparison of three physiologically based pharmacokinetic models of benzene disposition, Toxicol. Appl. Pharmacol. 110 (1991) 79–88.
[18] F.Y. Bois, M.T. Smith, R.C. Spear, Mechanisms of benzene carcinogenesis: application of a physiological model of benzene pharmacokinetics and metabolism, Toxicol. Lett. 56 (1991) 283–298.
[19] F.Y. Bois, E.T. Jackson, K. Pekari, M.T. Smith, Population toxicokinetics of benzene, Environ. Health Perspect 104 (Suppl. 6) (1996) 1405–1411.
[20] C.C. Travis, J.L. Quillen, A.D. Arms, Pharmacokinetics of benzene, Toxicol. Appl. Pharmacol 102 (1990) 400–420.
[21] K. Yeowell-O'Connell, N. Rothman, M.T. Smith, R.B. Hayes, G. Li, S. Waidyanatha, M. Dosemeci, L. Zhang, S. Yin, N. Titenko-Holland, S.M. Rappaport, Hemoglobin and albumin adducts of benzene oxide among workers exposed to high levels of benzene, Carcinogenesis 19 (1998) 1565–1571.
[22] K. Yeowell-O'Connell, N. Rothman, S. Waidyanatha, M.T. Smith, R.B. Hayes, G. Li, W.E. Bechtold, M. Dosemeci, L. Zhang, S. Yin, S.M. Rappaport, Protein adducts of 1,4-benzoquinone and benzene oxide among smokers and nonsmokers exposed to benzene in China, Cancer Epidemiol. Biomarkers Prev. 10 (2001) 831–838.
[23] S.M. Rappaport, K. Yeowell-O'Connell, M.T. Smith, M. Dosemeci, R.B. Hayes, L. Zhang, G. Li, S. Yin, N. Rothman, Non-linear production of benzene oxide-albumin adducts with human exposure to benzene, J. Chromatogr. B 778 (2002) 367–374.
[24] S.M. Rappaport, S. Waidyanatha, Q. Qu, R. Shore, X. Jin, B. Cohen, L.C. Chen, A.A. Melikian, G. Li, S. Yin, H. Yan, B. Xu, R. Mu, Y. Li, X. Zhang, K. Li, Albumin adducts of benzene oxide and 1,4-benzoquinone as measures of human benzene metabolism, Cancer Res. 62 (2002) 1330–1337.
[25] N. Rothman, G.L. Li, M. Dosemeci, W. Bechtold, G.E. Marti, Y.Z. Wang, M. Linet, L.Q. Xi, W. Lu, M.T. Smith, N. Titenko-Holland, L.P. Zhang, W. Blot, S.N. Yin, R.B. Hayes, Hematotoxocity among Chinese workers heavily exposed to benzene [see comments], Am. J. Ind. Med. 29 (1996) 236–246.
[26] Q. Qu, A.A. Melikian, G. Li, R. Shore, L. Chen, B. Cohen, S. Yin, M.R. Kagan, H. Li, M. Meng, X. Jin, W. Winnik, Y. Li, R. Mu, K. Li, Validation of biomarkers in humans exposed to benzene: urine metabolites, Am. J. Ind. Med. 37 (2000) 522–531.
[27] T.A. McDonald, N.T. Holland, C. Skibola, P. Duramad, M.T. Smith, Hypothesis: phenol and hydroquinone derived mainly from diet and gastrointestinal flora activity are causal factors in leukemia, Leukemia 15 (2001) 10–20.
[28] S.M. Rappaport, Biological considerations in assessing exposures to genotoxic and carcinogenic agents, Int. Arch. Occup. Environ. Health 65 (1993) S29–S35.
[29] R.B. Hayes, S.N. Yin, M. Dosemeci, G.L. Li, S. Wacholder, L.B. Travis, C.Y. Li, N. Rothman, R.N. Hoover, M.S. Linet, Chinese Academy of Preventive Medicine, National Cancer Institute Benzene Study Group, Benzene and the dose-related incidence of hematologic neoplasms in China, J. Natl. Cancer Inst. 89 (1997) 1065–1071.
[30] D.C. Glass, C.N. Gray, D.J. Jolley, C. Gibbons, M.R. Sim, L. Fritschi, G.G. Adams, J.A. Bisby, R. Manuell, Leukemia risk associated with low-level benzene exposure, Epidemiology 14 (2003) 569–577.
[31] S.M. Rappaport, L.L. Kupper, Variability of environmental exposures to volatile organic compounds, J. Expo. Anal. Environ. Epidemiol. 14 (2004) 92–107.
[32] L. Wallace, Environmental exposure to benzene: an update, Environ. Health Perspect. 104 (Suppl. 6) (1996) 1129–1136.