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Protein adducts as dosimeters of human exposure to styrene, styrene-7,8-oxide, and benzene

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Abstract

Cysteinyl adducts of hemoglobin (Hb) and albumin (Alb) formed via reactions with reactive species were measured in 48 subjects exposed to styrene (0.24–55.2 ppm) and to styrene-7,8-oxide (SO) (2.65–107 ppb) in a factory producing boats in the USA. Hb and Alb adducts were also investigated among 88 workers exposed to benzene (0–138 ppm) in several Chinese factories. The particular adducts were *S*-(2-hydroxy-1-phenylethyl) cysteine, from reactions of SO with Alb (designated SO-Alb), and *S*-phenylcysteine, from reactions of the CYP450 benzene metabolite, benzene oxide (BO), with Hb and Alb (designated BO-Hb and BO-Alb, respectively). The relationships between adduct levels and exposures were investigated in both studies. The estimated slopes varied considerably among the particular combinations of adduct and agent to which the workers were exposed, ranging from 0.815 pmol BO-Hb/g Hb per ppm benzene to 24 400 pmol SO-Alb/g Alb per ppm SO. We used these estimated slopes, along with kinetic constants, to predict the systemic doses of SO and BO in humans per mg of styrene, SO or benzene per kg body weight, under certain assumptions. Using RX to signify the particular electrophile (SO or BO) the doses of RX to the blood per unit of dose varied between 2.21 and 4110 nM RX-h/mg agent per kg b.w. The dose of RX to the blood arising from inhalation of SO was almost 2000 times that of styrene (i.e. 4110 vs. 2.21 nM RX/mg agent per kg b.w.) and 430–781 times that of benzene (i.e. 4110 vs. 5.26–9.55 nM RX/mg agent per kg b.w.), depending upon the study. Comparable estimates of the blood dose of BO were obtained from adducts of Hb and Alb and two independent studies of BO-Alb yielded similar dose estimates. These results point to the utility of protein adducts as dosimeters of reactive electrophilic species in occupational studies. Finally, significant levels of background adducts of SO and BO with Hb and A1b were observed among workers, among control subjects and in commercial human proteins. Levels of these background adducts were too great to have arisen from non-occupational exposures to styrene or benzene or from cigarette smoking.

Keywords: Adducts; Benzene; Styrene; Styrene-7,8-oxide

Cysteinyl protein adducts have increasingly been used to investigate the disposition of reactive species, notably epoxides and quinones, resulting from human exposure (Gan et al., 1988; Calleman et al., 1993; Granath et al., 1996; Rappaport et

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al., 1996; Yeowell-O'Connell et al., 1996a, 1998; Lindstrom et al., 1998). The proteins most commonly used for this purpose are hemoglobin (Hb) and albumin (Alb), which are easily isolated from blood and purified in large quantities. Hb and Alb adducts have significant advantages over DNA adducts (e.g. from lymphocytes) for human dosimetry. That is, Hb and Alb are much more abundant than DNA in blood; unlike DNA adducts, protein adducts are not repaired; and unlike lymphocytic DNA, the rate of turnover of Hb and Alb are well understood (Granath et al., 1992).

Cysteinyl adducts of Hb and Alb are known to form via reactions with reactive species associated with occupational exposures to styrene and benzene, two commercially important chemicals to which there are significant occupational exposures (Yeowell-O'Connell et al., 1996a, 1998). Both chemicals are readily metabolized by CYP450 isozymes (primarily CYP2EI for benzene (Seaton et al., 1994; Valentine et al., 1996) and CYP2B6 and CYP2E1 for styrene (Nakajima et al., 1994; Kim et al., 1997)). As shown in Figs. 1 and 2, the major products of human metabolism are the reactive epoxides, benzene oxide (BO) and

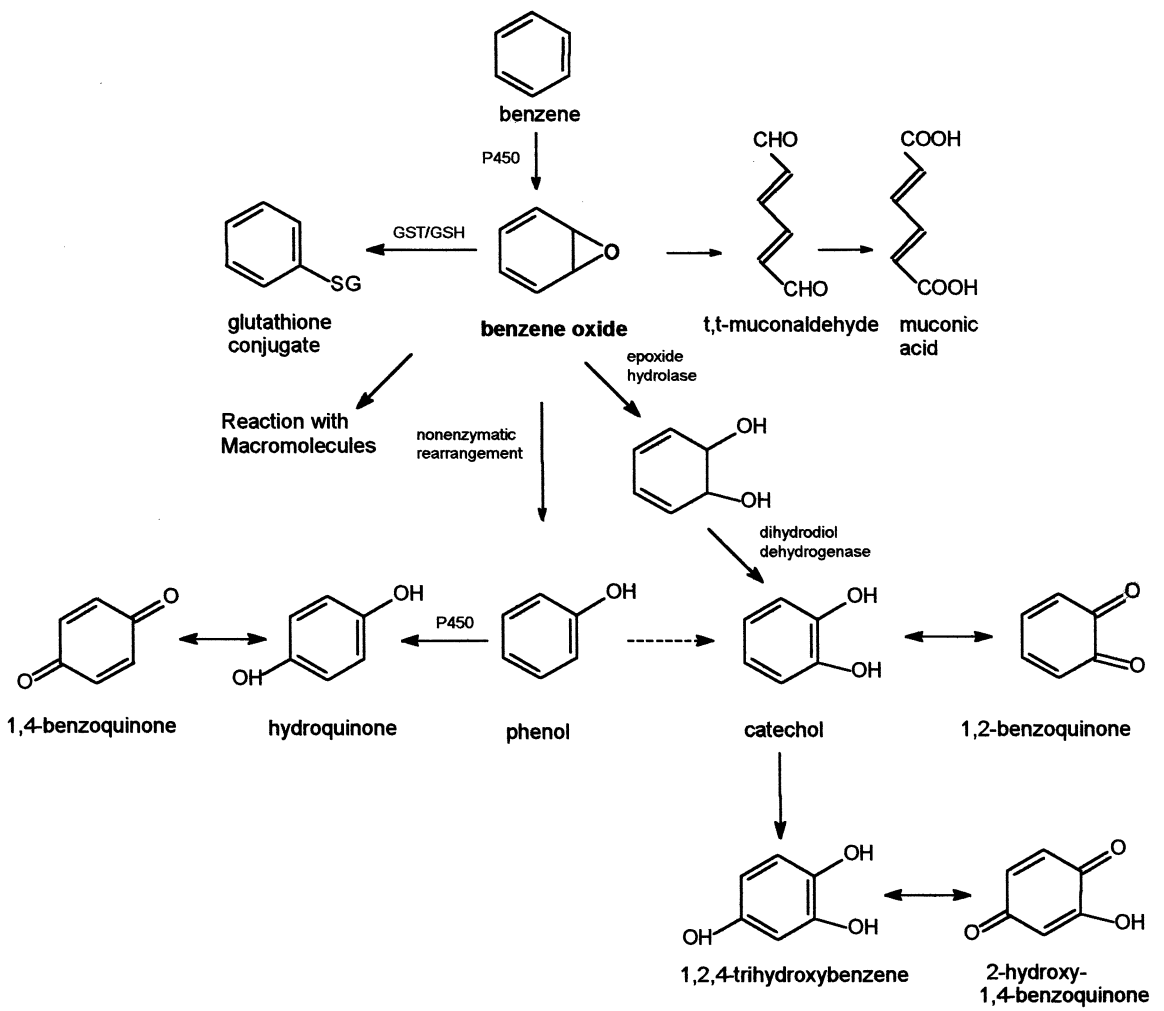

Fig. 1. Scheme for human metabolism of benzene. Note that all metabolites are derived from benzene oxide.

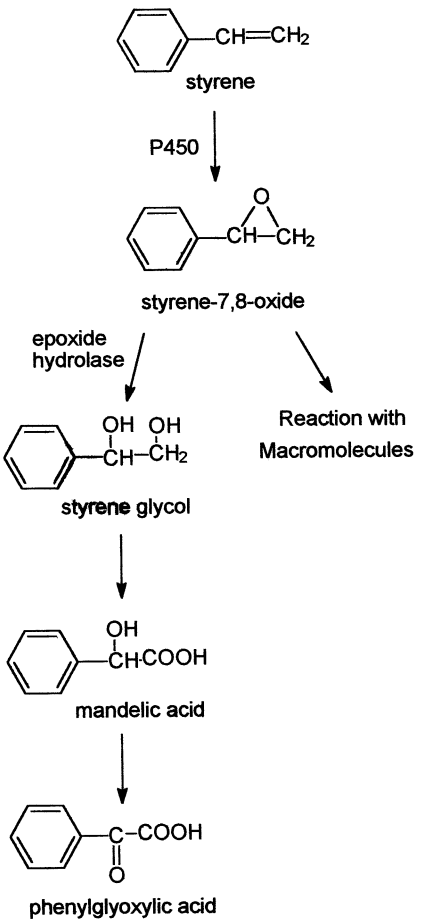

Fig. 2. Scheme for human metabolism of styrene. Styrene-7,8-oxide can also be directly absorbed into the body upon inhalation.

styrene-7,8-oxide (SO), which produce adducts and also give rise to other metabolites.

Direct exposure to SO has also been documented in the reinforced plastics industry following oxidation of styrene during the manufacturing process (Fjeldstad et al., 1979; Pfäffli et al., 1979; Pfäffli and Säämänen, 1993; Yeowell-O'Connell et al., 1996a). Thus, adducts of SO can arise from either direct inhalation of the vapor or indirectly from CYP450 metabolism of styrene. We have previously reported results of Alb and Hb adducts of both SO (designated SO-Alb and SO-Hb, respectively) and BO (designated BO-Alb and BO-Hb, respectively) in occupationally-exposed workers (Yeowell-O'Connell et al., 1996a, 1998). BO-Alb and BO-Hb were both associated with benzene exposure, while only SO-Alb (and not SO-Hb) was correlated with exposure to styrene and SO. In addition, preliminary evidence suggested that direct exposure to SO was about 2000 times more effective than exposure to styrene (with subsequent metabolism to SO) at producing Alb adducts of SO in human blood (Rappaport et al., 1996).

In this paper we will use previously determined levels of SO-Alb, BO-Alb and BO-Hb (Yeowell-O'Connell et al., 1996a, 1998; Rappaport et al., 1996) to estimate the blood doses of SO and BO following occupational exposure to styrene and SO in the reinforced plastics industry and to benzene in several industries where benzene was used as a solvent. We will then compare these doses, per unit of exposure to the agent inhaled. Finally, we will examine and discuss background adduct levels measured among unexposed individuals and in human commercial proteins.

1. Kinetic basis for protein adducts as dosimeters

The kinetic relationships used for evaluating tissue doses from levels of protein adducts are derived largely from the work of Ehrenberg and co-workers (Ehrenberg et al., 1974; Osterman-Golkar et al., 1976; Ehrenberg and Hussain, 1981). Let [RX] represent the blood concentration of electrophile RX in vivo following exposure and/or metabolism of a precursor molecule. Let D_{RX} represent the blood dose, defined as the integrated blood level of RX over time t which is given by

$$D_{RX} = \int_0^t [RX](t)\,dt = \frac{[RX]_0}{k_e}(1 - e^{-k_e t}) \qquad (1)$$

where $[RX]_0$ represents the level of RX at time $t = 0$ and k_e is the pseudo-first-order elimination rate constant representing loss of RX by all reactions. RX can react with any nucleophile Y (at concentration [Y]) in the blood, to produce adduct RY (in our work Y represents a free cysteine thiol residue of Hb or Alb) according to the following reaction:

$$RX + Y \xrightarrow{k_{RX\text{-}Y}} RY + X \quad (2)$$

where $k_{RX\text{-}Y}$ represents the second-order reaction rate constant, which is generally estimated from in vitro experiments (Ehrenberg and Hussain, 1981). Assuming that adduct RY is stable in vivo, then the dose of RX (in units of concentration × time, e.g. nM RX-h) to protein Y, designated, ${}_{Y}D_{RX}$, can be predicted from the adduct level as follows:

$${}_{Y}D_{RX} = \frac{[RY]}{[Y]k_{RX\text{-}Y}} \quad (3)$$

When workers are chronically exposed to RX, [RY] rises and falls over time due to production of adduct, associated with current exposure, and loss of adduct, due to protein turnover and/or adduct instability. Let μ_{RX} and μ_{RY} represent the mean values of [RX] and [RY] in the blood of a worker exposed to RX according to a consistent regimen such as 8 h/day, 5 days/week. If RY is chemically stable, then μ_{RX} can be related to μ_{RY}, with knowledge of the rate of protein turnover, which differs between Hb and Alb. Hb is removed with old erythrocytes from the blood; this occurs at a given erthrocyte age (designated t_{er}) for each animal species; e.g. $t_{er} \cong 120$ days in humans (Granath et al., 1992). In this case

$$\frac{\mu_{RX\text{-}Hb}}{[Hb]} = k_{RX\text{-}Hb} \cdot \mu_{RX} \cdot \frac{t_{er}}{2} \quad (4)$$

from which

$${}_{Hb}D_{RX} = \mu_{RX} \cdot \frac{t_{er}}{2} = \frac{\mu_{RX\text{-}Hb}}{[Hb] \cdot k_{RX\text{-}Hb}} \quad (5)$$

From Eq. (5), the Hb dose of RX is numerically equivalent to the mean level of RX in the blood times one half of the erythrocyte lifetime (about 60 days in humans). In contrast, Alb is eliminated by a random process so that

$$\frac{\mu_{RX\text{-}Alb}}{[Alb]} = \frac{k_{RX\text{-}Alb} \cdot \mu_{RX}}{k_{Alb}} \quad (6)$$

where k_{Alb} represents the first-order rate of turnover of Alb, approximately 0.0014 h^{-1} in humans (Peters, 1970). Thus, the dose of BO to Alb can be predicted from levels of BO-Alb as

$${}_{Alb}D_{RX} = \frac{\mu_{RX}}{k_{Alb}} = \frac{\mu_{RX\text{-}Alb}}{[Alb] \cdot k_{RX\text{-}Alb}} \quad (7)$$

Here we see that the dose of RX to serum Alb is equal to the mean level of RX in the blood times $1/k_{Alb}$, representing the Alb turnover time constant (about 30 days in humans).

2. Methods

2.1. Air and blood sampling

Individual airborne exposures to styrene (0.24–55.2 ppm) and SO (2.65–107 ppb) were estimated by repeated personal sampling (over 1 year) among 48 workers of both sexes in a reinforced plastics factory (producing boats) in the USA (Yager et al., 1993; Yeowell-O'Connell et al., 1996a).

Between two and seven personal air samples for styrene were obtained from each of the 48 reinforced plastics workers whereas one to three personal air samples for SO were obtained from 20 of the workers. SO-Alb and SO-Hb were assayed in one to three blood samples from each of the 48 reinforced plastics workers.

Among benzene-exposed workers, five personal air samples (0–138 ppm) and a single blood specimen were obtained from 88 workers of both sexes in several Chinese factories during a 2 week survey period (Rothman et al., 1996). BO-Hb was measured in 83 workers while BO-Alb was measured in 38 workers (Yeowell-O'Connell et al., 1998).

2.2. Measurement of protein adducts

SO adducts [as *S*-(2-hydroxy-1-phenylethyl) cysteine] were analyzed by releasing the adduct from the protein using Raney Ni and then derivatizing to yield the pentafluorobenzoyl ester of 2-phenylethanol (Rappaport et al., 1996).

BO-Alb and BO-Hb (as *S*-phenylcysteine) were analyzed by simultaneously cleaving and derivatizing the adduct with trifluoroacetic anhydride plus methanesulfonic acid, yielding phenyltrifluorothioacetate (Yeowell-O'Connell et al., 1996b). Both assays employed gas chromatography-mass spectrometry with negative ion chemical ionization (GC-NICI-MS) to measure these fluorinated derivatives.

2.3. Data analysis

Worker-specific average exposure levels and SO-Alb levels were aggregated by job and then used for multiple linear regression analysis with SO-Alb as dependent variable and styrene exposure and SO exposure as independent variables (Rappaport et al., 1996). Levels of SO-Hb were not related to exposure (Yeowell-O'Connell et al., 1996a) and therefore were not used in the dosimetric analysis described in that paper.

The relationship between benzene exposure and adduct levels employed multiple linear regression analysis with the worker-specific level of BO-Alb or BO-Hb as dependent variable and the worker-specific benzene exposure (the geometric mean of the five air samples) plus covariates (respirator use was the only significant covariate) as independent variables (Yeowell-O'Connell et al., 1998). All statistical analyses were conducted with SAS software (SAS Institute, Cary, NC, USA).

2.4. Background adducts

Sources of background adducts were investigated by measuring SO-Alb, BO-Alb and BO-Hb in Hb and Alb obtained from unexposed controls, SO-Alb from 15 blood donors and hospital staff in Italy (Fustinoni et al., 1998), BO-Alb from 19 and BO-Hb from 42 individuals employed either in a sewing machine manufacturing plant or an administrative facility in China, and in commercial human Hb and Alb from Sigma Chemical Co. (St. Louis, MO, USA), which represent pooled blood proteins from presumably unexposed persons in the USA (Yeowell-O'Connell et al., 1998). Samples of these proteins were assayed using the same procedures as employed for the workers blood specimens. Estimated mean adduct levels were compared between proteins using *t*-tests with unequal variances and two-tailed probabilities.

3. Results

3.1. Dosimetry

The linear relationships between adduct levels and job-specific exposures (styrene and SO) or worker-specific exposures (benzene) were investigated. The parameters, estimated from multiple linear regression analyses, are summarized in Table 1. The estimated slopes varied considerably among the particular combinations of adduct and agent to which the workers were exposed, ranging from 0.815 pmol BO-Hb/g Hb per ppm benzene to 24400 pmol SO-Alb/g Alb per ppm SO (Rappaport et al., 1996; Yeowell-O'Connell et al., 1998).

We used these estimated slopes and Eq. (5) or Eq. (7) to predict the systemic doses of SO and BO in humans per mg of styrene, SO or benzene per kg body weight. In estimating the exposure dose of the airborne contaminant, we assumed a ventilation rate of 1 m^3/h, a body weight of 50, 60, or 65 kg (depending upon the sex and race of the workers), a retention factor of 0.97 for styrene

Table 1
Parameters estimated from linear regressions of adduct levels on personal exposures to styrene, SO or benzene

Agent	Adduct	Number of workers	Intercept (SE) (pmol adduct/g protein)	Slope (SE) (pmol adduct/g protein/ppm Agent)	Reference
Styrene	SO-Alb	48	750 (180)	11.4 (5.3)	Rappaport et al., 1996
SO	SO-Alb	48	750 (180)	24400 (6540)	Rappaport et al., 1996
Benzene	BO-Hb	83	35.3 (2.0)	0.815 (0.17)	Yeowell-O'Connell et al., 1998
Benzene	BO-Alb	38	130 (30)	15.4 (3.2)	Yeowell-O'Connell et al., 1998

Table 2
Doses of SO or BO predicted from slopes listed in Table 1

RX	Agent	Y	$k_{RX\text{-}Y}$[a] (l g $Y^{-1}h^{-1}$)	Body weight (kg)	DOSE ($_YD_{RX}$) (nM RX-h per mg agent/kg b.w.)	Reference
SO	Styrene	Alb	4.8×10^{-4}	65	2.21	Rappaport et al., 1996
SO	SO	Alb	4.8×10^{-4}	65	4,110	Rappaport et al., 1996
BO	Benzene	Hb	0.177×10^{-4}	60	5.26	Yeowell-O'Connell et al., 1998
BO	Benzene	Alb	5.19×10^{-4}	60	6.82	Yeowell-O'Connell et al., 1998
BO	Benzene	Alb	5.19×10^{-4}	55	9 55	Lindstrom et al., 1998; Bechtold et al., 1992

[a] Second order reaction rate constants estimated in references Rappaport et al., 1993 (SO) and Lindstrom et al., 1998 (BO).

(Petreas et al., 1995) and SO or of 0.48 for benzene (Nomiyama and Nomiyama, 1974), and an occupational regimen of 8 h/day and 5 days/week for either 30 days (Alb) or 60 days (Hb). For example, among Chinese workers exposed to 0–138 ppm benzene we found a slope of 15.4 pmol BO-Alb/g Alb per ppm benzene (Table 1), which represents an estimate of $\frac{\mu_{BO\text{-}Alb}}{[Alb]}$ per ppm benzene exposure. Assuming $k_{BO\text{-}Alb} = 5.19 \times 10^{-4}$ l/g Alb-h (Lindstrom et al., 1998) and exposure for 8 h/day and 5 days/week for the $1/k_{Alb} = 4.25$ weeks prior to blood collection from a 60 kg worker, we employed Eq. (7) to estimate a dose of 6.82 nM BO-h/mg benzene per kg body weight. That is,

$$
\begin{aligned}
{}_{Alb}D_{BO} &= \frac{[BO\text{-}Alb]}{[Alb]\cdot k_{BO\text{-}Y}} \\
&= \frac{15.4\ \text{pmol}}{\text{g Alb-ppm}} \times \frac{\text{nmol}}{10^3\ \text{pmol}} \times \frac{\text{g Alb-h}}{5.19\times10^{-4}\ \text{l}} \\
&\times \frac{\text{ppm}}{3.2\ \text{mg m}^{-3}} \\
&\times \frac{60\ \text{kg}}{(1\ \text{m}^3\ \text{h}^{-1})\times(8\ \text{h day}^{-1})\times(5\ \text{day week}^{-1})} \\
&\times 4.25\ \text{week} \times 0.48\ \text{(ret.)} \\
&= \frac{6.82\ \text{nMBO-h}}{\text{mg kg}^{-1}\ \text{b.w.}}
\end{aligned}
$$

The result of all such calculations are summarized in Table 2 along with one obtained from a similar study of BO-Alb among workers exposed to benzene (4.4–23 ppm) (Bechtold et al., 1992); in that case we assumed a body weight of 55 kg (Chinese females) and a positive bias of 1.87, to account for the fact that the proteins had not been dialyzed in that study (Lindstrom et al., 1998). The calculations indicate a wide range of doses per unit of administered dose with $2.21 \le {}_YD_{RX} \le 4110$ nM RX-h/mg agent per kg body weight.

3.2. Background adducts

From Table 1 it is clear that the intercepts of the linear relationships between adduct levels and exposure were all highly significant. This suggests that the linear models predicted high levels of SO_{Alb} (750 pmol/g), BO-Hb (35.3 pmol/g) and BO-Alb (130 pmol/g) at an exposure level of zero. To further elucidate this finding, samples of blood from unexposed controls and commercial human Alb and Hb were assayed for SO-Alb, BO-Alb or BO-Hb. The results, shown in Table 3, indicate that the levels of adducts in the controls and commercial proteins were at or above those estimated as the intercepts from the workers investigated in the two studies.

4. Discussion

This study points to several interesting observations. First, it is clear that exposure to SO is much more effective at producing Alb adducts than is exposure to either styrene or benzene. Indeed, as shown in Table 2, the dose of RX to the blood arising from inhalation of SO was almost 2000 times that of styrene (i.e. 4110 vs. 2.21 nM RX/mg agent per kg b.w.) and 430–781 times that of

benzene (i.e. 4110 vs. 5.26–9.55 nM RX/mg agent per kg b.w.), depending upon the study. This reflects the fact that, upon inhalation, SO rapidly enters the blood where it can react with nucleophiles, whereas styrene and benzene require metabolic activation followed by transport of RX to the blood where reactions with Hb and Alb can take place. (Although some adduction of Alb can occur within the hepatocyte, where Alb is synthesized, experiments with Sprague Dawley rats dosed with styrene or SO indicate that virtually all SO-Alb was formed in the blood (Rappaport et al., 1993)). Similar results were reported by Granath et al. (1996) who observed that inhalation of ethene was only 1/200-th as effective as that of ethylene oxide at producing hydroxyethylvaline adducts of human Hb. Thus, we conclude that direct inhalation of volatile epoxides will generally lead to much greater systemic doses of RX (per unit of exposure) than inhalation of the precursor molecules.

Since SO has been observed in the air of reinforced-plastics factories, investigators should consider the possibility that similar in situ oxidation reactions (i.e. generating epoxides) result from other polymerization processes, involving, for example, ethylene or butadiene. Given the mutagenicity and/or carcinogenicity of ethylene oxide, butadiene mono- and di-epoxides, and other epoxides, emissions of even small amounts of such biologically active substances into the air could have important health consequences.

The calculations, summarized in Table 2, indicate that comparable estimates of the blood dose of BO were obtained from adducts of Hb and Alb in the same blood samples, suggesting that either protein can be used as a dosimeter of BO in the blood. However, BO is about 29 times more reactive with cysteinyl residues of human Alb than of human Hb (as indicated by the values of $k_{RX\text{-}Y}$ shown in Table 2); and, after accounting for the rates of protein turnover, the ratio of $\mu_{RX\text{-}Alb}$ to $\mu_{RX\text{-}Hb}$ (from Eqs. 4 and 6) at a given level of exposure should be 14.5. This indicates that the long term mean level of Alb adducts in a person's blood should be substantially greater than that of Hb adducts at given level of exposure. Thus, for practical purposes it would generally be preferred to employ BO-Alb rather than BO-Hb as a dosimeter in human studies. From Table 2 we also see that similar dose estimates were derived from levels of BO-Alb measured by Bechtold et al. (1992), with adjustment (Lindstrom et al., 1998), and from our study (Yeowell-O'Connell et al., 1998). Since completely different methods of air and biological monitoring were used in these studies, the similarity of results offers some validation to the dosimetric estimates.

Given that we expect 11.4 and 24400 pmol SO-Alb/g per ppm of exposure to styrene and SO, respectively, the intercepts of the exposure-SO_{Alb} relationships, summarised in Table 1, point to background adducts in this population equivalent to 66 ppm styrene or 31 ppb SO. Likewise, given that we expect 0.815 pmol BO-Hb/g and 15.4 pmol BO-Alb/g per ppm of benzene exposure, the intercepts of 35.3 pmol BO-Hb/g Hb and 130 pmol BO-Alb/g Alb would suggest unknown exposures equivalent to 8.4–43 ppm benzene. Such high ambient concentrations of styrene and benzene are clearly unrealistic, based upon published levels of non-occupational exposure which are typically a few ppb among non smokers (Wallace, 1989; Fishbein, 1992), and alternative explana-

Table 3
Background levels of SO-Alb, BO-Alb and BO-Hb in unexposed workers, controls and commercial human proteins

Protein Source	SO-Alb (SE) (pmol/g)	BO-Alb (SE) (pmol/g)	BO-Hb (SE) (pmol/g)
Workers (intercept)	750 (180, $n = 48$)[a]	130 (30, $n = 38$)[b]	35.3 (2.0, $n = 83$)[b]
Controls	2740 (261, $n = 15$)[c]	107 (5.51, $n = 19$)[b]	34.2 (1.60, $n = 42$)[b]
Commercial	1180 (68, $n = 78$)	99.2 (10.5, $n = 19$)	52.3 (3.6, $n = 19$)

[a] From Rappaport et al., (1996).
[b] From Yeowell-O'Connell et al., (1998).
[c] From Fustinoni et al., (1998).

Table 4
Background levels of SO-Alb, BO-Alb and BO-Hb in smoking versus non-smoking control subjects

Protein source	SO-Alb (SE) (pmol/g)	BO-Alb (SE) (pmol/g)	BO-Hb (SE) (pmol/g)
Smoking controls	3490 (826, $n = 3$)[a]	94.1 (7.76, $n = 6$)[b]	33.5 (1.95, $n = 19$)[b]
Non-smoking controls	2540 (365, $n = 12$)[a]	113 (6.93, $n = 13$)[b]	34.8 (2.50, $n = 23$)[b]
P-value of difference	0.384	0.119	0.686

[a] From Fustinoni et al. (1998).
[b] From Yeowell-O'Connell et al. (1998).

tions must be sought. Even though styrene and benzene are known constituents of tobacco smoke (Wallace, 1989; Fishbein, 1992), the reported levels of smokers' benzene and styrene exposures are still much lower than those predicted from the observed background adducts. Indeed, as shown in Table 4, the background levels of SO-Alb, BO-Hb and BO-Alb were large enough to obscure any differences between smokers and non-smokers among the control subjects. Thus, we conclude that cigarette smoking contributed relatively little to the production of adducts observed in these studies.

One possibility is that the intercepts shown in Table 1 are artifacts of biased (attenuated) exposure-adduct relationships, due to statistical measurement errors associated with the independent variables. However, as shown in Table 3, estimates of the background levels observed in control subjects and in commercial human Hb and Alb were at or above the estimated intercepts. This tends to rule out the possibility that the intercepts were artifactual. It is also possible that the adduct assays were themselves biased, in the sense that the protein matrix could have generated interfering analyses during the procedures. Investigations of this prospect are ongoing.

Finally, the background adducts could represent unknown exposures or endogenous production of reactive species that can react with proteins to produce the same analytes in our assays. Given the high levels of background adducts compared to those in workers exposed to extremely large quantities of styrene or benzene, such exogenous or endogenous exposures could be quite important, and our data suggest that they vary among populations. That is, by comparing adduct levels of the commercial proteins (derived from blood donors in the USA) with those of the controls (derived from Italian (SO-Alb) or Chinese subjects (BO adducts)) a significant difference was observed for SO-Alb (1180 vs. 2740 pmol/g, $P < 0.001$) and BO-Hb (52.3 vs. 34.2 pmol/g, $P = 0.019$) but not for BO-Alb (99.2 vs. 107 pmol/g, $P = 0.53$).

This analysis shows how cysteinyl protein adducts can be used to investigate human exposures to epoxides or other biologically reactive intermediates, that cannot be easily assayed in blood or other tissues. Such applications are of obvious value in applications to control hazardous exposures in the workplace. Furthermore, by employing the dosimetric relationships given in Eqs. (3)–(6), protein adducts can also be used to compare the doses of toxic metabolizes per unit of administered dose in humans and animals (Granath et al., 1996; Rappaport et al., 1996; Lindstrom et al., 1998; Yeowell-O'Connell et al., 1998). This allows great insight to be gained into the metabolism of procarcinogens, at different administered doses among animal species, that can be used for risk assessment and other regulatory purposes. Our work also demonstrates how protein adducts can be used to investigate background exposures to toxic substances arising from dietary and endogenous sources which do not lend themselves to direct investigation.

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