On the Importance of Exposure Variability to the Doses of Volatile Organic Compounds

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The connection between occupational exposure to volatile organic compounds (VOCs) and the resulting internal doses is complicated by variability in air levels from day to day and by nonlinear kinetics of metabolism. We investigated long-term liver doses of VOCs and their metabolites using a physiologically based toxicokinetic model, to which 10,000 random 8-h exposures were inputted. Three carcinogenic VOCs were studied (i.e., benzene, perchloroethylene, and acrylonitrile); these compounds are all bioactivated in the liver and represent a wide range of an important toxicokinetic parameter \(\left(\frac{V_{\max}}{K_{M}}\right)\). For each VOC, simulations were performed using mean air concentrations (\(\mu_{X}\)) between 0.0003 and 1 mg/l (which covers both linear and saturated metabolism) and using coefficients of variation of exposure (\(CV_{X}\)) between 0.23 and 2.18 (which includes most occupational settings). Two long-term measures of internal dose were examined, i.e., the area under the liver concentration-time curve (\(AUCL\)) and the area under the metabolic rate-time curve (\(AURC\)). Interestingly, both \(AUCL\) and \(AURC\) were linear functions of cumulative exposure (\(CE\), mg h/l air) even when metabolism was saturated and \(CV_{X}\) was large. Yet, at a given \(CE\), both \(AUCL\) and \(AURC\) were affected by \(CV_{X}\), with the magnitude of the effect increasing with \(\frac{V_{\max}}{K_{M}}\) (i.e., perchloroethylene < benzene < acrylonitrile). Nonetheless, the effects of \(CV_{X}\) were typically only a few percent and should be of little consequence unless a VOC has large values of \(\frac{V_{\max}}{K_{M}}\) \(\mu_{X}\), and \(CV_{X}\). We conclude that \(CE\) should be a sufficient predictor of the dose of either the parent chemical (VOC) or its metabolite in the liver, even when metabolism is nonlinear. We also observed that \(AUCL\) and \(AURC\) were sensitive to changes in values of model parameters in the high-variability scenarios, suggesting that (when \(CV_{X}\) is large) the population variability of \(AUCL\) and \(AURC\) can be quite large at a fixed \(CE\).

Key Words: volatile organic compounds; benzene; perchloroethylene; acrylonitrile; variation of exposure.

If exposures are of sufficient intensity and duration, critical tissues are damaged and workers suffer adverse health effects. Here, we are interested in the relationship between airborne exposure and the dose of a contaminant accumulated by a worker over many years. Thus, we only consider chronic effects of exposure and not acute, allergic, or reproductive effects, where the timing as well as the magnitude of exposures can be important.

As a worker breathes a contaminant at a given air level (mg/l), he or she takes up some portion of the chemical in the lungs. The uptake is related to the breathing rate (l/h) and the retention, a dimensionless quantity that depends upon the physical and chemical properties of the contaminant (gaseous or particulate, solubility, particle size, etc.). Thus, during a brief period of time, uptake = (air level) \(\times\) (breathing rate) \(\times\) retention, with units of mg/h. Once the contaminant is cleared from the lungs, it can be distributed to tissues and eliminated by a host of excretory and metabolic processes. The difference between input and output gives rise to an internal mass, or burden (mg), of the substance at a particular time. From mass-balance considerations, the rate at which the burden changes during a brief period is

\[
\frac{d(burden)}{dt} = \text{uptake} - (\text{elimination rate}) \times \text{burden}
\]

where the elimination rate has units of h\(^{-1}\). By integrating the burden over time, the internal dose can be derived, where dose = \(\int_{0}^{t} \text{burden}(t) \, dt\), with units of mg-h. The internal dose can be defined more conventionally as the area under the tissue concentration–time curve, i.e., \(AUC = \int_{0}^{t} \text{burden}(t) \, dt\), with units of mg-h/l. Since the internal dose ultimately determines the extent of tissue damage, our ability to relate workers’ exposures to the corresponding \(AUC\) values is fundamental to understanding and preventing occupational diseases.

The connection between exposure and \(AUC\) is complicated by the intermittent nature of the occupational regimen, where workers tend to be exposed for 8 h per day, and by the profound variability in air levels occurring from one workday to another. Occupational exposures typically vary 15-fold from day to day within workers (median value), and variation greater than 70-fold is observed in about a fourth of occupational groups (Kromhout et al., 1993). Given such great variability, it is
EXPOSURE VARIABILITY AND DOSES OF VOLATILE ORGANIC COMPOUNDS

MATERIALS AND METHODS

Toxicokinetic model. Figure 1 displays a physiologically based toxicokinetic model that is widely accepted as a reasonable depiction of mammalian absorption and elimination of VOCs (Andersen, 1981a; Droz and Guillemine, 1983; Ramsey and Andersen, 1984). The input to the model is the contaminant, at air concentration \( X \) (mg/l), inhaled into a central (lungs/blood) compartment at the alveolar ventilation rate \( Q_{Alv} \) (lh). The contaminant is absorbed into the arterial blood according to its blood-air partition coefficient \( \lambda_a \). Once inside the body, the chemical is transported at the rate of the cardiac output \( Q_{Car} \) (lh) via the arterial blood at concentration \( C_{Art} \) (mg/l). The chemical is distributed to parallel tissue groups, consisting of the liver (\( L \), the only metabolizing tissue), the rapidly perfused tissues (\( RPT \), mainly the central organs), the slowly perfused tissues (\( SPT \), mainly muscles and skin), and the fat (\( F \)), at rates defined by the perfusion rates \( Q_{RPT} \) for the liver, etc. (lh). The tissue groups are all assumed to be homogenous well-mixed volumes. Transfer of the chemical from the arterial blood to each tissue group is governed by the tissue-blood partition coefficient \( \lambda_{t,t} \) and volume \( (V_t) \). The concentration is designated as \( C_t \) (mg/l) for the liver, etc. The chemical is cleared from the body either passively, by exhalation at rate \( \frac{C_{Art}Q_{Alv}}{L} \) (mg/h), or by metabolism in the liver to metabolite \( M \) at rate \( R_M \) (mg/h). For benzene, perchloroethylene, and acrylonitrile, note that \( M \) would represent the initial epoxide produced by cytochrome P450 metabolism, namely, benzene oxide, perchloroethylene epoxide, and cyanohydrin oxide, respectively.

The nonlinear behavior of the model shown in Figure 1 relates to liver metabolism, which obeys Michaelis-Menten kinetics at rate \( R_M = \frac{C_{Art}Q_{Alv}}{L} \lambda_a \), where \( V_{max} \) represents the maximum rate of metabolism (mg/h) and \( K_M \) (mg/l) is the

![FIG. 1. Physiologically-based toxicokinetic model for volatile organic compounds. Legend: \( C_{Art} \), arterial blood concentration; \( C_{Vox} \), venous blood concentration; \( Q_{Alv} \), alveolar ventilation rate; \( Q_{Car} \), cardiac output; \( Q_{RPT} \), blood flow rate through the gth tissue group for \( g = RPT \) (rapidly perfused tissues); \( SPT \) (slowly perfused tissues); \( F \) (fat) and liver (\( L \)); \( \lambda_a \), blood-air partition coefficient; \( \lambda_{t,t} \), tissue-blood partition coefficient; \( V_t \), volume for the gth tissue group; \( C_t \), concentration of parent compound in the gth tissue group; \( R_M \), rate of metabolism; \( M \), burden of metabolite(s). Units: concentrations in mg/l, ventilation and blood-flow rates in l/h, volumes in l, \( R_M \) in mg/h, \( M \) in mg.]

reasonable to ponder whether day-to-day fluctuations in air levels might alter the relationship between exposure and internal dose. If air levels vary greatly from day to day (about some mean value), would the \( AUC \) differ from that observed when the air level is the same (mean) value each day? This subject has received only limited attention (Kumagai and Matsunaga, 1995; Rappaport, 1985, 1991; Roach, 1966, 1977; Smith, 1987; Smith, 1992).

Logically, exposure variability can affect the relationship between exposure and internal dose only if two conditions are met (Rappaport, 1991). First, the contaminant must be eliminated from the body sufficiently rapidly so as not to accumulate from week to week. This is because substances that accumulate (notably insoluble dusts, heavy metals, and lipophilic organic compounds) achieve burdens much greater than the mass taken up in a single day and thereby are reasonably invariant to daily fluctuations in air levels. For such contaminants, cumulative exposure \( (CE) \), i.e., the product of the mean exposure and time (with units of mg-h/l), should be a valid predictor of the long-term internal dose \( (AUC) \). Second, the contaminant must be either taken up by, or eliminated from, the body by a nonlinear process over the relevant range of exposure. This condition is necessary because linear kinetics would maintain a strict proportionality between \( AUC \) and \( CE \) even when the contaminant is rapidly absorbed and eliminated (a restatement of ‘Haber’s Law’) (Cox, 1995; Olson and Cumming, 1981; Rappaport, 1991).

Volatile organic compounds (VOCs) are rapidly eliminated via nonlinear (saturable) metabolism. Since many VOCs have been associated with chronic health effects, the purpose of this investigation is to explore the influence of exposure variability upon the internal doses of these compounds and their metabolites. Points will be illustrated with three chemicals that are known or suspected human carcinogens, namely, benzene (Hayes et al., 1997; Savitz and Andrews, 1996, 1997; Snyder, 2002), perchloroethylene (Lash and Parker, 2001), and acrylonitrile (Collins and Strother, 1999; Kirman et al., 2000). These substances were chosen because they are biotransformed in the liver by phase-I metabolism and possess an important toxicokinetic parameter \( \left( \frac{V_{max}}{Q_{Car}\lambda_a} \right) \) to be defined that ranges in value from low (perchloroethylene), to moderate (benzene), to high (acrylonitrile). The carcinogenicity of all three compounds is likely due to the action of one or more reactive metabolites. The sites of tumor formation include the hematopoietic system (benzene), liver and kidney (perchloroethylene), and the brain (acrylonitrile).

In what follows, we will couple a random time series of simulated air levels, representing the variability in occupational exposure over many years, with a physiologically based toxicokinetic model, representing the disposition of VOCs and metabolite production in the body. Such toxicokinetic models provide the means to relate external exposure to internal levels and, thus, are well suited for evaluating the doses of VOCs and their metabolites following prolonged periods of occupational exposure.
liver-blood concentration ($\frac{C_l}{C_b}$) of the chemical at which $R_M = V_{max}/2$. If ($\frac{C_l}{C_b} < K_M$, then metabolism is pseudo-first order (linear) at rate $R_M \approx \frac{V_{max} (C_l/2)}{K_M}$. If ($\frac{C_l}{C_b} > K_M$, then metabolism is zero order at rate $V_{max}$. At intermediate values of ($\frac{C_l}{C_b}$), the rate of metabolism lies between these limiting values. We also identify the dimensionless quantity $\frac{V_{max}}{V_{max}}$, representing “the maximum concentration gradient that exists across the liver at a given blood flow” divided by $K_M$ (Andersen, 1981b). When $\frac{V_{max}}{V_{max}} < 1$, metabolism is not complete at low substrate concentrations, and the transition from first- to zero-order behavior is gradual. Conversely, when $\frac{V_{max}}{V_{max}} > 1$, metabolism is essentially complete at low substrate concentrations, and this transition is abrupt (Andersen, 1981b).

Model parameters and simulation. The flow rates (l/h) and tissue volumes (l) were scaled to a 70-kg human working at 50 W of exercise according to Tardif et al. (2002), i.e., $Q_{SS} = 1323$, $Q_{L\to B} = 603$, $Q_L = 96.4$, $Q_F = 36.2$, $Q_{SPF} = 163$, $Q_{SPF} = 307$, $V_L = 1.82$, $V_F = 13.3$, $V_{RPT} = 3.50$, and $V_{SPF} = 40.6$. The chemical-dependent partition coefficients and biochemical constants, shown in Table 1, were compiled from (Dobrev et al., 2001; Sweeney et al., 2003; Travis et al., 1990) after scaling $V_{max}$ for a 70-kg human as $V_{max} = V_{max} \cdot 70^{0.75}$, where $V_{max}$ is the scaling coefficient given by the authors.

Simulation involved introducing a time series of 8-h occupational exposures $\{X_t\}$ into the model shown in Figure 1, where $X_t$ (mg/l) is the air concentration during the $t^{th}$ 8-h workday ($i = 1, \ldots, 10,000$). We modeled $X_t$ as a lognormal variate with true (unobservable) mean $\mu_X$ and variance $\sigma_X^2$ (Rappaport, 1991); by randomly sampling from a given distribution (defined by $\mu_X$ and $\sigma_X^2$), we simulated 10,000 occupational exposures representing about 40 years of work. Simulations were performed with $\mu_X = 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0$ mg/l, and (for each value of $\mu_X$) values of $CV_X = \frac{\sigma_X}{\mu_X}$ equal to 0.23, 0.62, and 2.18. These $CV_X$ values were selected to represent levels of occupational-exposure variability ranging from “very low” to “moderate” to “very high.” 

For reference, these $CV_X$ values correspond, respectively, to the 2nd, 41st and 84th percentiles of the cumulative distribution of geometric standard deviations reported by Kromhout et al. (1993). Note that, for the lognormal variate $X_t$, $CV_X = \sqrt{e^{\sigma_X^2} - 1}$, where $\sigma_X^2$ is the variance of $Y_t = \ln(X_t)$, and the geometric standard deviation $= e^{\frac{\sigma_X}{2}}$. Since we consider only a single worker in our simulations, $\sigma_X^2$ represents the within-person variance component from Kromhout et al.] After 10,000 simulated workdays, the cumulative exposure (mg-l/h) was calculated as $CE = 8 \times \sum_{i=1}^{10,000} X_i$, for 8 h of exposure per workday.

The toxicokinetic model employed mass-balance equations to describe the rates of change of the chemical concentration in each compartment, based largely upon relationships given by Ramsey and Andersen (1984) (see Supplementary Data). These equations were solved numerically by Euler’s method, using a program written with SAS software (SAS Institute, Cary, NC) and employing a time step $\Delta t$ of either 0.01 h (benzene and perchloroethylene) or 0.002 h (acrylonitrile), as required to maintain continuity. Following 8 h of simulated exposure at air concentration $X_t$, the inhaled air concentration was reduced to zero, and liver concentrations were monitored until $t = 336$ h to ensure complete clearance of the chemical from all tissues. The time series $\{X_t\}$ produced the corresponding time series of internal doses, represented by the AUC of the parent chemical in the liver after each exposure $\{AUCL_i\}$ (mg-h/l), where $AUCL_i = \sum_{j=1}^{\Delta t} C_{LV} \cdot \Delta t$ and $C_{LV}$ is the liver concentration (mg/l), after the $t^{th}$ exposure step for the $i^{th}$ exposure. The long-term liver dose of parent chemical after 10,000 exposures was calculated as $AUCL = \sum_{i=1}^{10,000} AUCL_i$, (mg-h/l), and the corresponding long-term dose of metabolite as $AURC = \sum_{i=1}^{10,000} AURC_i$ (mg). Even though both $AUCL$ and $AURC$ are long-term dose metrics, note that they are dimensionally different.

We focused our models upon the liver and metabolite doses ($AUCL$ and $AURC$) even though the liver is not necessarily the target of toxicity for the VOCs investigated. Because our model only permits metabolism to take place in the liver, the liver is more sensitive to perturbations in levels of the parent compound than are the blood and other tissue groups. Thus, effects of exposure variability upon $AUCL$ are at least as great as upon the analogous $AUC$ values for other tissues. Likewise, $AURC$ represents a global measure of metabolite production that should be relevant to all tissues where metabolites are ultimately distributed from the liver by the systemic circulation.

Sensitivity analysis. Additional simulations were conducted to determine the sensitivity of the two dose metrics $AUCL$ and $AURC$ to each of the parameters in the model. Each parameter was increased by 1% and the full simulation was repeated for 4 exposure distributions, representing a wide range of mean values ($\mu_X = 0.0003$ and 0.3 mg/l) and variability ($CV_X = 0.23$ and 2.18). While testing sensitivity to blood flow rates, mass balance was maintained by reducing $Q_{SPF}$, as necessary, to compensate for the 1% increase in $Q_{SPF}, Q_L$, or $Q_F$. Normalized sensitivity coefficients were estimated as the percentage change observed in $AUCL$ or $AURC$ divided by the 1% change in the parameter of interest (e.g., a 2% increase in $AUCL$ or $AURC$ would correspond to a normalized sensitivity coefficient of 2). 

RESULTS

Exposure-Dose Relationships for Benzene

To illustrate the effect of exposure variability on the daily liver doses of benzene and its metabolite at a given mean exposure, values of $\{AUCL\}$ and $\{AURC\}$ are plotted versus exposure $\{X_t\}$ in Figure 2 when the mean exposure $\mu_X = 0.010$ mg/l (3.13 ppm) and $CV_X = 0.23$ (very low variability) or $CV_X = 2.18$ (very high variability). The effect of saturable metabolism is apparent when $CV_X = 2.18$, but not when $CV_X = 0.23$; this is due to the much greater range of benzene air levels in the high-variability scenario, relative to the mean value of 0.010 mg/l. Note that the shapes of the nonlinear relationships in Figures 2A and 2B differ when $CV_X = 2.18$. As benzene metabolism approaches saturation, the ratio of the arterial blood concentration to the exposure concentration $\frac{C_{AVL}}{X_t}$ increases, and a larger fraction of benzene is

| TABLE 1 Chemical-Dependent Parameters for the Toxicokinetic Model Shown in Figure 1 |
|---------------------------------|-----------------|-----------------|-----------------|
| Partition coefficients:         | Benzene$^a$     | Perchloroethylene$^b$ | Acrylonitrile$^c$ |
| $\lambda_B$                     | 7.4             | 10.3            | 154             |
| $\lambda_L$                     | 1.49            | 5.90            | 1.51            |
| $\lambda_{RPT}$                 | 1.49            | 5.90            | 1.34            |
| $\lambda_{SPF}$                 | 2.03            | 3.10            | 1.16            |
| $\lambda_F$                     | 54.9            | 119             | 0.942           |

Biochemical constants:

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<th>Value</th>
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<td>$V_{max}$, mg/h</td>
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<tr>
<td>$K_M$, mg/l</td>
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</tr>
<tr>
<td>$Q_L$, $\mu_X$</td>
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Note. $\lambda_B$ is the blood-air partition coefficient and $\lambda_B$ is the tissue-blood partition coefficient for the $g^{th}$ tissue group [for $g$ = rapidly perfused tissues (RPT), slowly perfused tissues (SPT), fat (F), and liver (L)]. $V_{max}$ is the maximum rate of metabolism scaled to a 70-kg human, and $K_M$ is the Michaelis-Menten constant.

$^a$From Travis et al. (1990).

$^b$From Dobrev et al. (2002).

$^c$From Sweeney et al. (2003).

$^d$Assumes that $Q_L = 96.4$ l/h.
distributed to the fat, where it is stored pending eventual release to the systemic circulation (and the liver) during the subsequent period of zero exposure. This ultimately leads to values of AUCLi that are disproportionally greater than those observed at lower exposure levels. Thus, the relationship between AUCLi and exposure concentration exhibits concave-upwards behavior that becomes pronounced when $X_i/C_21$ $0.05$ mg/l (Fig. 2A). At the same time, saturation of benzene metabolism leads to reduced uptake and increased passive clearance of benzene. These effects combine to disproportionably reduce metabolism during periods of high benzene exposure and give rise to the concave-downwards shape of the relationship between AURCi and exposure (Fig. 2B). Accordingly, the rate of metabolite production is substantially saturated, given exposure to benzene in the range of 0.2–0.4 mg/l (63–126 ppm).

The relationships shown in Figure 2 suggest that large variability in exposure can increase the liver dose of benzene and reduce the corresponding dose of the benzene metabolite at a given mean exposure $\mu_X$. This is illustrated in Figure 3, which shows the time series of daily liver doses {AUCLi} and the corresponding long-term liver dose (AUCL) (Figs. 3A and 3B) as well as the daily metabolite doses {AURCi} and the corresponding long-term metabolite dose (AURC) (Figs. 3C and 3D), when $\mu_X = 0.010$ mg/l and $CV_X = 0.23$ (very low variability, Figs. 3A and 3C) or $CV_X = 2.18$ (very high variability, Figs. 3B and 3D). The figures illustrate that, indeed, after 10,000 simulated workdays, AUCL and AURC (shown at the right axis) are different when $CV_X = 0.23$ ($AUCL = 3.9$ g·h/l, $AURC = 205$ g) than when $CV_X = 2.18$ ($AUCL = 4.4$ g·h/l, $AURC = 185$ g). Yet, the two values of AUCL and AURC only differ by 10–13%, despite the enormous differences in the variability of the two time series {AUCLi} and {AURCi} giving rise to the long-term doses.

The relationships shown in Figure 3 indicate that AUCL and AURC increase linearly with workday when $\mu_X = 0.010$ mg/l, regardless of the magnitude of $CV_X$; that is, the slope for AUCL versus workday [in (mg·h/l)/d] = $39\mu_X$ when $CV_X = 0.23$, and equals $44\mu_X$ when $CV_X = 2.18$, while the slope for AURC versus workday (in mg/d) = $2050\mu_X$ when $CV_X = 0.23$ and equals $1850\mu_X$ when $CV_X = 2.18$. In fact, the same results were observed for both AUCL and AURC over an extremely wide range of benzene exposures representing kinetics that changed from linear ($\mu_X = 0.0003$ mg/l, 0.1 ppm) to fully saturated ($\mu_X = 1.0$ mg/l, 313 ppm) (results not shown). Since CE is a linear function
of time, i.e., \( CE = \mu_X \times t \) for \( t \) representing exposure time in h (\( t = 80,000 \) h in our simulations), then \( CE \) must be a good predictor of the long-term internal dose of benzene (\( AUCL \)) or its metabolite (\( AURC \)), regardless of the variability in air levels from day to day (\( CV_X \)). Figure 4 shows graphs of \( AUCL \) and \( AURC \) versus \( CE \) for benzene exposures when \( \mu_X = 0.001 \) or 1.0 mg/l and \( CV_X = 0.23 \) or 2.18. Clearly, linear relationships are observed in both cases with intercepts equal to zero and slopes of either \( AUCL / CE \) (Figs. 4A and 4B) or \( AURC / CE \) (Figs. 4C and 4D). (Since \( CE = \mu_X \cdot t \) and since \( AUCL = k \cdot \mu_X \cdot t \), then \( AUCL \) is a constant multiple of \( CE \) and \( k = AUCL / CE \). The same argument holds for \( AURC \)). Furthermore, after 10,000 simulated workdays, \( AUCL \) and \( AURC \) are very close in value for both the low- and high-variability scenarios, indicating that exposure variability had small effects upon \( AUCL / CE \) and \( AURC / CE \) for benzene when \( 0.001 \leq \mu_X \leq 1 \) mg/l.

**Effects of Exposure Variability upon \( AUCL / CE \) and \( AURC / CE \)**

It was illustrated in Figures 4A and 4C that, when the toxicokinetics for benzene were linear (\( \mu_X = 0.001 \) mg/l), \( AUCL / CE \) and \( AURC / CE \) were virtually unchanged for scenarios involving either very low variability (\( CV_X = 0.23 \)) or very high variability (\( CV_X = 2.18 \)). However, when benzene metabolism was saturated (\( \mu_X = 1.0 \) mg/l), the slopes differed marginally between scenarios; that is, \( AUCL / CE \) increased from 9.45 when \( CV_X = 0.23 \) to 9.75 when \( CV_X = 2.18 \) (Fig. 4B), while \( AURC / CE \) concurrently decreased from 64.6 to 52.5 l/h (Fig. 4D). The same behavior was observed for simulations involving perchloroethylene and acrylonitrile, although the magnitudes and patterns of the deviations differed (results not shown). This indicates that, at a given \( CE \), highly variable exposure distributions can lead to marginally different internal doses (\( AUCL \) and \( AURC \)) than those of low variability.

The effects of exposure variability on the slopes \( AUCL / CE \) and \( AURC / CE \) (after 10,000 8-h workdays) are summarized in Figures 5A–5F for benzene, perchloroethylene, and acrylonitrile when \( 0.0003 \leq \mu_X \leq 1.0 \) mg/l and when \( CV_X = 0.23, 0.62, \) and 2.18. For each chemical, \( AUCL / CE \) and \( AURC / CE \) are hardly affected by exposure variability when \( \mu_X \leq 0.01 \) mg/l, even when \( CV_X = 2.18 \). However, as \( \mu_X \) increases above 0.01 mg/l, upwards divergence of \( AUCL / CE \) was observed (Figs. 5A, 5C, and 5E) along with downwards divergence of \( AURC / CE \) (Figs. 5B, 5D, and 5F), consistent with increasing saturation...
of VOC metabolism. These changes occur first for $CV_X = 2.18$ and then for $CV_X = 0.62$ and 0.23, respectively.

The curves represented by $CV_X = 0.23$ and 0.62 in Figure 5 are very similar, suggesting that low or moderate variation in occupational exposure has little impact upon the relationship between either $AUCL/CE$ and $\mu_X$ or $AURC/CE$ and $\mu_X$. However, deviations of the high-variability curves ($CV_X = 2.18$) from the low-variability curves ($CV_X = 0.23$) can be large, depending on the particular VOC and on whether $AUCL$ or $AURC$ is being considered. Here we define the high-variability deviation for $AUCL/CE$ as $\frac{(AUCL/CE)_{high var} - (AUCL/CE)_{low var}}{(AUCL/CE)_{low var}}$, and that for $AURC/CE$ as $\frac{(AURC/CE)_{high var} - (AURC/CE)_{low var}}{(AURC/CE)_{low var}}$. These high-variability deviations (in percent) are shown in Figure 6A and Figure 6B for $AUCL/CE$ and $AURC/CE$, respectively, for the three VOCs in our study. Referring first to $AUCL/CE$, the maximum deviations differed among the VOCs, i.e., 2% for perchloroethylene, 21% for benzene, and 570% for acrylonitrile (Fig. 6A). On the other hand, maximum deviations of the $AURC/CE$ were very uniform across the VOCs, ranging between $-29$ and $-31$%. Note that these maximum deviations occurred at values of $\mu_X$ between 0.1 and 0.3 mg/l, depending upon the particular VOC. Since the mean exposures corresponding to maximum deviations were on the cusps between linear and saturable metabolism, the low-variability scenario resulted in essentially linear metabolism on all workdays; here, $AUCL/CE$ and $AURC/CE$ were near the baseline values observed at much lower mean exposures (see Fig. 5). On the other hand, the high-variability scenario included many days where metabolism was in the saturable range, thereby producing major deviations in $AUCL/CE$ and $AURC/CE$ from their near-baseline values. For example, from Figure 6A, the maximum deviation in $AUCL/CE$ for acrylonitrile occurred when $\mu_X = 0.1$ mg/l. Referring to Figure 5E, we see that, when $\mu_X = 0.1$ mg/l and $CV_X = 0.23$, $AUCL/CE = 5.4$, a value only marginally greater than the baseline value of 3.8 observed when $\mu_X \leq 0.003$ mg/l. Yet, when $\mu_X = 0.1$ mg/l and $CV_X = 2.18$, $AUCL/CE = 36.5$, a 7-fold deviation from the low-variability scenario! As $\mu_X$ increased above 0.1 mg/l for acrylonitrile, metabolism shifted into the saturable range, and $AUCL/CE$ increased dramatically with $\mu_X$ when $CV_X = 0.23$; this narrowed the gap in $AUCL/CE$ between the low- and high-variability scenarios.

**Effects of $\frac{V_{max}}{Q_{L}}$ on $AUCL/CE$ and $AURC/CE$**

The deviations in $AUCL/CE$ and $AURC/CE$ between high-variability and low-variability scenarios increased in the following order: perchloroethylene < benzene < acrylonitrile, which matches the order of the toxicokinetic parameter $\frac{V_{max}}{Q_{L} K_{M}}$ identified earlier. When $\frac{V_{max}}{Q_{L} K_{M}} > 1$, the transition from first- to zero-order kinetics is abrupt, being described as ‘flip-flop’ behavior (Andersen, 1981b). As a consequence, the toxicokinetics of
FIG. 5. Relationships between the slope representing long-term liver dose versus cumulative exposure (AUCL/CE, dimensionless) and long-term metabolite dose versus cumulative exposure (AURC/CE, l/h) and the mean exposure $\mu_X$ (mg/l) to benzene (A and B), perchloroethylene (C and D), and acrylonitrile (E and F), following 10,000 simulated occupational exposures (80,000 total hours of exposure). The coefficient of variation of exposure CVX = 0.23 (solid curve), 0.62 (small dashed curve), or 2.18 (large dashed curve).
Conversely, efficient metabolism leads to a much larger
(AUCL/CE) for acrylonitrile at baseline than for perchloroethy-
ene (Fig. 5E), compared to 53.6 for perchloroethylene (Fig. 5C).

The picture regarding the metabolite dose was different, given
maximum deviations in \(\text{AURC/CE} \) of about \(-30\%\) for all three
VOCs (Fig. 6B). This is because the large baseline value of
\(\text{AURC/CE} \) for acrylonitrile tended to offset the abrupt reduction
in daily metabolite dose (\(\text{AURC}_i\)) occurring during the high-
exposure days. Since benzene and perchloroethylene do not
exhibit flip-flop kinetics, their reductions in \(\text{AUCL}_i\) or \(\text{AURC}_i\)
were more modest during the high-exposure days; but these
changes were offset by their smaller baseline values of
\(\text{AURC/CE} \), yielding essentially the same percent deviations as
for acrylonitrile.

**Sensitivity Analysis**

Results of the sensitivity analyses are summarized in Figure 7
for \(\text{AUCL} \) and in Figure 8 for \(\text{AURC} \), based upon a 1% increase
in each of the toxico-kinetic parameters. The sensitivities of the two
dose metrics were greatly influenced by the variability of expo-
sure (\(\text{CV}_X\)) at a given mean exposure (\(\mu_X\)). That is, long-term
doses were much more sensitive to changes in model parameters
when \(\text{CV}_X = 2.18\) than when \(\text{CV}_X = 0.23\). Indeed, it was common
to observe normalized sensitivity coefficients greater than \(\pm 5\)
when \(\text{CV}_X = 2.18\), whereas coefficients rarely exceeded \(\pm 2\)
when \(\text{CV}_X = 0.23\). This suggests that \(\text{AUCL} \) and \(\text{AURC} \)
would vary considerably across a population exposed at a
given \(\mu_X\) when exposure was highly variable under either linear
(\(\mu_X = 0.0003 \text{ mg/l} \)) or saturated (\(\mu_X = 0.3 \text{ mg/l} \)) kinetics.
The normalized sensitivity coefficients shown in Figures 7 and 8
indicate general sensitivity to most parameters. This probably
reflects the structure of the model, where all parameters, except
the partition coefficients and \(K_M\), were functions of body weight
and, therefore, were highly correlated. In comparing among
VOCs, the most notable difference concerns sensitivity of \(\text{AUCL} \)
in the high-exposure, high-variability scenario (Fig. 7D), where
deviations were negative for acrylonitrile but were positive for
benzene and perchloroethylene. This probably points to the
lipophobic nature of acrylonitrile (whereas the other compounds
are lipophilic), because days of saturating exposure would not
lead to a buildup of acrylonitrile in the fat (with subsequent
release to the circulation and the liver) but rather to increased
passive clearance in the exhaled air.

Regarding perturbations to the parameters that influence
metabolic clearance, i.e., \(V_{\text{max}}, K_M\), and \(Q_L\), \(\text{AUCL} \) was
consistently more sensitive for benzene and acrylonitrile (high-
affinity substrates) than for perchloroethylene (low-affinity
substrate) (see Fig. 7). This suggests that factors affecting
blood flow to the liver (such as exercise rate) and those influen-
cing metabolism (such as genetic polymorphisms as well as
enzyme induction and inhibition) would affect liver doses of

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**FIG. 6.** High-variability deviations in either the slope representing long-
term liver dose versus cumulative exposure (\(\text{AUCL/CE} \), dimensionless) (A) or
the slope representing long-term metabolite dose versus cumulative exposure
(\(\text{AURC/CE} \), l/h) (B) for a given mean exposure \(\mu_X \) (mg/l) [based upon
simulations involving 10,000 occupational exposures to benzene, perchloro-
ethylene, and acrylonitrile (80,000 total hours of exposure)].
FIG. 7. Normalized sensitivity of the long-term liver dose (AUCL) observed for a 1% increase in each parameter in the toxicokinetic model for various combinations of the mean exposure and the variation of exposure (A–D). Legend: $\mu_X$ is the mean exposure, $CV_X$ is the coefficient of variation of exposure, Perc is perchloroethylene, and ACN is acrylonitrile. Model parameters are defined in the caption for Figure 1.
FIG. 8. Normalized sensitivity of the long-term metabolite dose (AUC) observed for a 1% increase in each parameter in the toxicokinetic model for various combinations of the mean exposure and the variation of exposure (A–D). Legend: $\mu_X$ is the mean exposure, $CV_X$ is the coefficient of variation of exposure, Perc is perchloroethylene, and ACN is acrylonitrile. Model parameters are defined in the caption for Figure 1.
benzene and acrylonitrile to a much greater extent than they would for the dose of perchloroethylene, particularly in situations where exposure is highly variable from day to day.

**DISCUSSION**

Recognition that the dose of a substance ultimately determines its toxicity has been attributed to Paracelsus in work published more than 400 years ago (Gallo, 2001). Since then, the fields of toxicology and epidemiology have embraced the dose-response relationship as fundamental to the understanding of risks of diseases caused by chemical exposures. Unfortunately, our ability to estimate long-term doses from occupational exposures has been hampered by the variability in levels within and between persons in a given population and by the lack of historical measurements of exposure. In the face of large variability and few measurements, the ability to accurately quantify dose-response relationships is sadly limited. If exposure databases are to improve for future investigations, we must adopt sampling strategies that place a premium upon longitudinal exposure data collected according to sound statistical principles (Rappaport, 1991; Rappaport et al., 1995). A strong motivation for such a change would be to accept by default the premise that CE is the principal determinant of the long-term internal dose received by each person in a population. However, this acceptance presumes that internal dose will be effectively proportional to $\mu_X$ during some period of interest, regardless of the variability in exposure levels encountered from day to day (Rappaport et al., 1995).

The purpose of this paper has been to evaluate the premise that CE is a sufficient predictor of the internal doses of benzene, perchloroethylene, and acrylonitrile, three carcinogenic VOCs that are cleared in part by saturable metabolism over a wide range of toxicokinetic behaviors (as reflected by differing values of $V_{\text{max}} / \mu_X$). We observed in all cases that both the long-term liver dose ($AUCL$) and the long-term metabolite dose ($AURC$) were essentially linear functions of CE over about 40 simulated years of occupational exposure, even when daily-dose increments ($AUCL_i$ and $AURC_i$) were saturated (e.g., see Fig. 4). Thus, despite the enormous range of daily exposures that can be observed in the workplace, the straight-line slope representing $AUCL / CE$ or $AURC / CE$ after several years is essentially fixed for an individual worker at a given mean exposure ($\mu_X$).

Despite the linear relationship between $AUCL$ or $AURC$ and CE for an individual worker, the corresponding relationship across a population could well be nonlinear if some workers have mean air levels in the saturable range. For example, in a population of workers heavily exposed to the three VOCs investigated here, we would anticipate a concave-downwards shape in the exposure-biomarker relationship across the population for any biomarker located ‘downstream’ from the initial metabolic step (see Fig. 2B). Indeed, such shapes have been reported for protein adducts and urinary metabolites of benzene (both downstream biomarkers) (Rappaport et al., 2002a,b; Waidyanatha et al., 2004), as well as for the mortality-CE curve for lymphohematopoietic cancers among benzene-exposed workers (Hayes et al., 1996).

Our results indicate that individual workers who experience the same CE could nonetheless have different long-term internal doses ($AUCL$ or $AURC$) if their individual levels of exposure variability (values of $CV_X$) differed greatly. The magnitude of such deviations would depend upon the particular dose metric. Since most VOCs are metabolized to toxic products, the more important effect of exposure variability concerns its relation to the internal metabolite dose ($AURC$). Here, our results indicate that differences in $AURC / CE$ (arising from different values of $CV_X$ across the population) should be small for VOCs, with maximum deviations in the range of about $-30\%$ as observed for benzene, perchloroethylene, and acrylonitrile (Fig. 6B). We conclude that assignment of metabolite doses to VOCs, based solely on CE, should not compromise estimation of exposure-response relationships. This conclusion is at odds with the observation of Collins et al. that the number of ‘peak’ exposures to benzene (greater than 100 ppm) was a better predictor of lymphohematopoietic cancers than was CE, and could point to the large uncertainties in estimation of individual CEs mentioned by the authors (Collins et al., 2003). Another recent study found no evidence that the risk of lymphohematopoietic cancers was influenced by peak exposures (Glass et al., 2003).

Turning now to the liver dose of a VOC per se, our results indicate that $AUCL / CE$ can differ by several hundred percent between high- and low-variability scenarios, but only when $\mu_X$ and $CV_X$ are large and when $V_{\text{max}} / Q_l K_w \gg 1$. If not all three of these conditions are met, then deviations of $AUCL / CE$ should only be a few percent (see Fig. 6A for acrylonitrile when $\mu_X \leq 0.003$ mg/l or $CV_X \leq 0.62$ and for benzene and perchloroethylene at all values of $\mu_X$). Yet there could well be situations where such a nexus of three independent factors could occur. For example, if a worker was exposed to acrylonitrile in intermittent outdoor operations, giving rise to a large $\sigma_X^2$ (Kromhout et al., 1993), at a mean exposure level $\mu_X = 0.008$ mg/l, we would anticipate a deviation in $AUCL / CE$ of about $100\%$ from that of a coworker having the same $\mu_X$ but low-to-moderate exposure variability (see Fig. 6A). This mean air concentration ($\mu_X = 0.008$ mg/l) is about twice the 2004 Threshold Limit Value (2 ppm = 4.3 mg/m$^3$) (ACGIH, 2004) and, thus, would be unacceptable by current norms. However, exposures of this magnitude could easily escape detection in the developed world, where workplace air monitoring is sporadic at best, and could be commonplace in much of the developing world. Thus, we recommend that VOCs be screened to identify chemicals with $V_{\text{max}} / Q_l K_w > 2$, where exposure variability might lead to significant deviations in the long-term liver dose of parent chemical at a given CE. Of 16 VOCs reviewed in our perusal of the recent toxicokinetics literature, 5 chemicals had estimated values of $V_{\text{max}} / Q_l K_w > 2$. 
Sensitivity analyses indicated that AUCL and AURC were both sensitive to small changes in the toxicokinetic parameters when CV_x was large (see Figs. 7 and 8). This suggests that populations of workers exposed to a given mean exposure could have quite different long-term liver and metabolite doses of VOCs when exposure variability is great, due to differences in toxicokinetic parameters among individuals. Since physiologically-based toxicokinetic models rarely consider exposure variability, which is often quite large for VOCs in occupational and environmental settings (Rappaport and Kupper, 2004), our results indicate that such simulations probably underestimate the true sensitivity of model predictions to variability in model parameters across a population.

Finally, it is worth reiterating the sentiments of Clewell et al. that physiologically based toxicokinetic models offer powerful tools for investigating complex exposure-dose-response relationships in living organisms (Clewell et al., 2002). While most such applications have focused upon interspecies extrapolations and modes of toxic action, our analyses suggest that such models also offer logical avenues for elucidating the particular effects of exposure variability upon these complex relationships.

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APPENDIX

Equations Describing the Disposition of an Inhaled Volatile Organic Compound in the Body

Following Ramsey and Andersen (1984), assuming a steady-state rate (mg h^{-1}) between alveolar air and alveolar blood, the following mass-balance equation holds (for abbreviations refer to Figure 1 in the text):

\[ Q_{Alv} \cdot X + Q_{Car} \cdot C_{Ven} = Q_{Alv} \left( \frac{C_{Art}}{\lambda_B} \right) + Q_{Car} \cdot C_{Art}. \]  \hspace{1cm} (A1)

Using (A1) and solving for \( C_{Art} \), we obtain

\[ C_{Art} = \frac{Q_{Alv} \cdot X + Q_{Car} \cdot C_{Ven}}{Q_{Car} + \left( \frac{Q_{Ven}}{\lambda_A} \right)}. \]  \hspace{1cm} (A2)

And, from the mass-balance equation

\[ Q_{Car} \cdot C_{Ven} = \sum_{g=1}^{G} Q_{g} \left( \frac{C_{g}}{\lambda_g} \right), \]

where the subscript \( g \) refers to the \( g \)th tissue group (richly perfused, slowly perfused, fat or liver). For \( g = 1, \ldots, 4 \), the concentration of chemical in the mixed venous blood is

\[ C_{Ven} = \frac{\sum_{g=1}^{G} Q_{g} \left( \frac{C_{g}}{\lambda_g} \right)}{Q_{Car}}. \]  \hspace{1cm} (A3)

For the \( g \)th nonmetabolizing tissue group (richly-perfused, slowly-perfused, or fat), the rate of change (mg h^{-1}) in the amount \( A_g (= V_g C_g) \) of chemical is

\[ \frac{dA_g}{dt} = V_g \frac{dC_g}{dt} = Q_g \left( C_{Art} - \frac{C_{g}}{\lambda_g} \right). \]  \hspace{1cm} (A4)

Finally, the metabolism of the chemical is assumed to take place exclusively in the liver according to Michaelis-Menten kinetics. The mass-balance equation determining the rate of change in chemical concentration in the liver is

\[ V_L \frac{dC_L}{dt} = Q_L C_{Art} - Q_L \left( \frac{C_L}{\lambda_L} \right) - \frac{V_{max} \left( \frac{C_L}{\lambda_L} \right)}{K_M + \left( \frac{C_L}{\lambda_L} \right)}. \]  \hspace{1cm} (A5)

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