

# Trichloroethene Levels in Human Blood and Exhaled Breath from Controlled Inhalation Exposure

Joachim D. Pleil,<sup>1</sup> Jeffrey W. Fisher,<sup>2</sup> and Andrew B. Lindstrom<sup>1</sup>

<sup>1</sup>National Exposure Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 USA; <sup>2</sup>Toxicology Division, Armstrong Laboratory, Wright-Patterson Air Force Base, Ohio 45433 USA

The organic constituents of exhaled human breath are representative of bloodborne concentrations through gas exchange in the blood/breath interface in the lungs. The presence of specific compounds can be an indicator of recent exposure or represent a biological response of the subject. For volatile organic compounds, sampling and analysis of breath is preferred to direct measurement from blood samples because breath collection is noninvasive, potentially infectious waste is avoided, the sample supply is essentially limitless, and the measurement of gas-phase analytes is much simpler in a gas matrix rather than in a complex biological tissue such as blood. However, to assess the distribution of a contaminant in the body requires a reasonable estimate of the blood level. We have investigated the use of noninvasive breath measurements as a surrogate for blood measurements for (high) occupational levels of trichloroethene in a controlled exposure experiment. Subjects were placed in an exposure chamber for 24 hr; they were exposed to 100 parts per million by volume trichloroethene for the initial 4 hr and to purified air for the remaining 20 hr. Matched breath and blood samples were collected periodically during the experiment. We modeled the resulting concentration data with respect to their time course and assessed the blood/breath relationship during the exposure (uptake) period and during the postexposure (elimination) period. Estimates for peak blood levels, compartmental distribution, and time constants were calculated from breath data and compared to direct blood measurements to assess the validity of the breath measurement methodology. Blood/breath partition coefficients were studied during both uptake and elimination. At equilibrium conditions at the end of the exposure, we could predict actual blood levels using breath elimination curve calculations and a literature value partition coefficient with a mean ratio of calculated:measured of 0.98 and standard error (SE) = 0.12 across all subjects. blood/breath comparisons at equilibrium resulted in calculated *in vivo* partition coefficients with a mean of 10.8 and SE = 0.60 across all subjects and experiments and 9.69 with SE = 0.93 for elimination-only experiments. We found that about 78% of trichloroethene entering the body during inhalation exposure is metabolized, stored, or excreted through routes other than exhalation. **Key words:** blood/breath measurement, breath sampling, partition coefficients, time constants, trichloroethene, uptake and elimination models. *Environ Health Perspect* 106:573-580 (1998). [Online 12 August 1998] <http://ehpnet1.niehs.nih.gov/docs/1998/106p573-580pleil/abstract.html>

The occupational or environmental exposure to organic compounds is a potential source of adverse acute or chronic health effects. To assess and quantify the degree of an exposure requires both environmental monitoring of the subject's surroundings and biological monitoring of the subject's bodily systems to assure that all routes of exposure are represented. In the medical community, the direct measurement of various substances and biomarkers in the blood is one of the primary diagnostic tools in current use. Thus, the presence of specific xenobiotic compounds in a human subject's blood is considered to unambiguously confirm a recent exposure (or biological response) to an environmental pollutant or to indicate a disease state of the individual. Because the organic constituents of exhaled human breath are representative of their bloodborne concentrations through gas exchange in the blood/breath interface in the lungs, a breath measurement could conceivably replace a direct blood measurement.

A classic example of this is the Breathalyzer test for inebriation from ethanol (1). Under the assumption that changes in the exposure profile, the metabolic activity, and the elimination processes are slow with respect to the distributive flow of blood in the body, the exhaled breath concentrations should accurately reflect the overall current blood and tissue concentrations, and the time course of the concentrations should be useful in assessing these processes.

Exhaled breath analysis has been explored over the past 10 years as a diagnostic technique for assessing exposure to volatile organic compounds (VOCs); an overview of such work is available in recent review articles by Wallace et al. (2,3), and a more general history of the use of breath measurement in medicine has been written by Phillips (4). Throughout most of this body of work, there has been a tacit assumption that the breath reflects blood, especially for the nonpolar VOCs, and that the

blood/breath equilibrium in the alveoli is instantaneous. The literature has many examples of exposure assessments based on breath analysis that are followed by the development or use of various models to estimate delivered dose, bloodborne concentrations, or compartmental distribution within the body. Though these works are too numerous for an all-inclusive list to be presented here, some well-known specific examples are the chloroform work of Chinery and Gleason (5) and Weisel et al. (6); the modeling work of Raymer et al. (7), Wallace et al. (8), McKone (9), and Andersen (10); and the perchloroethene paper by Aggazzotti et al. (11).

Some researchers have preferred sampling and analysis of breath over direct measurement from blood samples because breath collection is noninvasive, potentially infectious waste is avoided, and the measurement of gas-phase analytes is much simpler in a gas matrix than in a complex biological tissue like blood. Also, in contrast to blood, the sample supply of breath is essentially limitless and the human system is not perturbed by its collection. A variety of sampling and analysis methods have been developed to exploit these advantages, including the single breath canister (SBC) method (12,13) used

Address correspondence to J.D. Pleil, U.S. EPA, NERL (MD-44), S-239C Environmental Research Center Annex, 79 Alexander Drive, Research Triangle Park, NC 27711 USA.

We thank the anonymous subject volunteers for their generous participation in the exposure scenarios. We are grateful for the technical assistance from J. Bowyer, P. Kizakevich, M. McCartney, L. Van Hoose, and K. Hudnell. Special thanks go to the QA/QC and research personnel from ManTech, Research Triangle Park, NC, and Dayton, OH. Chamber facilities and operation, subjects, and medical personnel were provided by Research Triangle Institute under U.S. Army Medical Research and Development Command Project 4970-04, grant # DAMD17-91-C-1007. Samples and analytical confirmation for QA/QC standards for breath work were provided by ManTech Environmental Technology, Inc., under EPA contract 68-D0-0106. Blood analyses were performed by ManTech Environmental Technology, Inc., under Air Force contract # F-41624-96-C-9010.

This work has been funded by the U.S. EPA and the U.S. Air Force. It has been subjected to agency review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Received 29 December 1997; accepted 28 April 1998.

for this work. The important feature of our methodology is the ability to collect an individual alveolar breath with a practical sample-to-sample time resolution of 15 sec; this becomes crucial during times of rapid change in the exposure concentration profile or during the initial rapid elimination at the end of an exposure period. Some examples of this methodology have been published recently (14–17).

In this work we tested the hypothesis that exhaled breath analysis is comparable to venous blood analysis in assessing the time course of trichloroethene concentration resulting from a controlled inhalation exposure. Specifically, pairs of human subjects spent 24 hr in a “live-in” chamber, the first 4 hr of which they were exposed to 100 parts per million by volume (ppmv) trichloroethene, and the remaining time they were exposed to pure air. Venous blood samples and single alveolar breath samples were collected in matched pairs from each subject during the exposure (uptake) and postexposure (elimination) periods. Real-time monitoring of the chamber air provided confirmation of the trichloroethene concentration during the exposure and its absence during the elimination phase. This work was part of a larger study that included cognitive testing, real-time physiological monitoring for stress and biological functions, and blood and urine metabolites measurements.

## Experimental Methods

**Blood sampling and analysis.** Blood samples were collected and analyzed according to methodology as specified by a standard operating procedure (18). Briefly, blood is drawn from a venous (arm) catheter; a 200- $\mu$ l aliquot is immediately added to an amber vial containing 200  $\mu$ l sulfuric acid (to deactivate metabolic activity), 100  $\mu$ l distilled water, and 100  $\mu$ l methanol. Then, 2 ml of cold methyl *tert*-butyl ether (MTBE) is added, the vial is vortexed and centrifuged, and the MTBE layer is transferred to a gas chromatography (GC) liquid autosampler vial. The processed samples are sealed, flash-frozen in liquid nitrogen, and subsequently stored and shipped on dry ice to the laboratory. Analyses by GC were performed with an electron capture detector (GC-ECD) with an HP-5890 GC (Hewlett-Packard, Avondale, PA) and a Vocol 30-m  $\times$  0.53-mm-i.d. capillary column. Quantitative standards were prepared fresh each day from control blood matrices spiked from 1 to 100  $\mu$ g/l.

**Breath sampling and analysis.** The SBC sampling apparatus consists of an evacuated 1-liter canister fitted with a small Teflon tube used as a mouthpiece. As the subject

closed her lips on the tube and exhaled, she opened the canister valve and the breath was collected, filling the evacuated volume. The subject was instructed to begin sample collection at the “bottom” (or end) of a normal resting tidal breath to achieve an alveolar sample so that the tracheal dead volume was expelled well before the canister sample valve was opened. A detailed description of this procedure and an investigation of the alveolar nature of an SBC sample in contrast to other techniques is available (12).

Though subsequent laboratory analysis can be performed with any of a variety of GC-MS methods for air, for our purposes here, the standard EPA Method TO-14 (19) was employed. Briefly, each breath sample was transported to the laboratory and pressurized with a neutral gas, and a dilution factor was calculated based upon pre- and postpressurization absolute pressure. The analytical instrumentation was fully automated to extract an aliquot (5 ml) from the canister, cryogenically concentrate, thermally desorb, and then inject onto a capillary column, and then analyze with a mass spectrometer. Carbon dioxide assays of breath samples were performed also by GC-MS. Specifically, all analyses were performed with a Graseby-Nutech 3550A cryoconcentrator (Graseby-Nutech, Smyrna, GA) with a 16-canister autosampler interfaced to an ITS40 (Magnum) GC-MS ion trap instrument (Finnigan MAT, San Jose, CA). The analytical column was an XTI-5, 30 m  $\times$  0.25-mm i.d. with 1.0- $\mu$ m stationary phase (Restek Corp., Bellefonte, PA). Quantitation was achieved by using external standards; system linearity was confirmed over the sample range with 5-point calibration. Daily response factors and system integrity were determined via single point calibration standards and canister blanks. A minimum of 25% replicate analyses (of real samples) were performed to continually assess system precision. Calibration standards were independently prepared and assessed by our on-site contractor, ManTech Environmental Technology, Inc., using certified standards from Alphasgaz (Walnut Creek, CA) and Scott Specialty Gases (Plumsteadville, PA).

**Human subjects.** Subjects were volunteers with informed consent; they were recruited and selected under institutional procedures by Research Triangle Institute (Research Triangle Park, NC). In total, we collected a variety of breath samples from 9 healthy adult subjects and blood samples from 18 subjects, some to develop methods and others to assess and correlate potential biomarker responses (metabolites) for future detailed study. For this work, detailed data sets of paired blood and breath

samples were successfully collected from 3 male and 3 female subjects (see Table 1).

Medical procedures were supervised by a board-certified, licensed physician, and invasive medical procedures were performed by licensed medical personnel. For safety and additional experimental data, subjects were constantly monitored by electrocardiograph, for blood pressure, and for thoracic electrical impedance. Because of the small number of subjects studied, no attempt was made to investigate effects dependent on physiological parameters or sex.

**Exposure chamber and sampling logistics.** A male–female pair of subjects was studied in each of three exposure experiments. Prior to entering the exposure chamber, subjects were fitted with venous catheters and trained to self-administer breath samples. Preexposure blood and breath samples were then collected. Subjects were seated in the exposure chamber and fitted with free flowing respirators to breathe hospital-grade air while the chamber concentration was adjusted to 100 ppmv trichloroethene. At a signal from the study coordinator, the subject removed the mask and the exposure period was started. This was designated as experiment time ( $t_{exp} = -240$  min); the end of the exposure (and start of the elimination period) is thus designated as  $t_{exp} = 0$  min for data graphing and analysis purposes. To avoid logistical problems for blood sampling, the two subjects’ time schedules were staggered by 5 min.

During the exposure period, paired blood and breath samples were collected as closely as possible to -240, -210, -180, -120, -60, and 0 min. When possible, an additional breath sample was collected at time = +1 min. At time = 0, the subjects put on their masks and began breathing hospital-grade (clean) air to begin the elimination period; masks were removed at about 10 min after the chamber had been flushed with clean air and a trichloroethene-free baseline had been established. Blood and breath sample pairs were collected as closely as possible to 15, 30, 60, 120, 240, 360, 480, and 600 min. For some subjects, we collected additional breath samples at 2, 3, 5, and 12 min and also collected additional blood and breath sample pairs at 720, 840, and 2,640 min. The subjects were required

**Table 1.** Physiological parameters of subjects

Subject ID #	Sex	Age (years)	Height (in)	Weight (lb)
108	Female	29	65	148
208	Male	33	75	161
109	Female	24	68	145
209	Male	22	66	118
110	Female	28	65	139
210	Male	23	70	134

to stay in the chamber for only 24 hr total time; the samples at 2,640 min (44 hr) were follow-up samples from a return visit.

**Interpretation of concentration data.** The uptake and elimination of VOCs such as trichloroethene as measured in breath are presumed to follow a multiexponential behavior, with separate terms for hypothetical body compartments representing blood, highly perfused tissues, poorly perfused tissues, etc. This gas distribution concept is discussed in the physiologically based pharmacokinetic (PBPK) modeling literature (5–11); the specific version of the model (including mathematical derivations and assumptions) used for this work is based upon the article by Wallace et al. (8). A detailed discussion of the pragmatic aspects of modeling such data and a literature review of the background mathematics has been written by Pleil and Lindstrom (20).

The time-course data of blood and breath concentrations were modeled separately for the uptake and the elimination periods. During the exposure, the model takes the form

$$C(t) = fC_{air} \sum A_i [1 - e^{(-k_i t)}], \quad (1)$$

where  $C(t)$  is the breath or blood concentration at time  $t$  (note that the independent variable  $t = t_{exp} - 240$ ),  $fC_{air}$  is a factor proportional to the exposure concentration ( $C_{air}$ ) where  $f$  is the ratio of expired to inspired concentration at equilibrium,  $A_i$  is a constant indicating the capacity of the  $i$ th compartment (for this form of the equation  $\sum A_i = 1$ ), and  $k_i$  is the time constant of the  $i$ th compartment's uptake rate. When Equation 1 is applied to blood data, the term  $C_{air}$  is actually a composite parameter that includes an adjustment for the effective transfer of the gas phase to the blood (the blood/breath partition coefficient  $P$ )

that accounts for Henry's law. For this work we express concentration units in micrograms per liter and time in minutes. We can extract the value for  $f$  from empirically determined uptake data from

$$f = C(t = \infty) / (C_{air} \sum A_i). \quad (2)$$

During the elimination period, starting with  $t = t_{exp} = 0$ , the concentration decay takes the form:

$$C(t) = fC_{air} + \sum A_i e^{(-k_i t)}, \quad (3)$$

where the definitions are similar to those for Equation 1; however, the modeled parameters  $A_i$  and  $k_i$  refer to elimination kinetics and are not necessarily the same values as their uptake counterparts. The design of the experiment sets the parameter for inspired air during the elimination period to zero ( $fC_{air} = 0$ ).

For the uptake portion of the experiments, the maximum blood and breath values can be estimated by evaluating the respective optimized model functions at  $t = 240$  (the end of the exposure period); approaching from the opposite time direction, the modeled elimination curves can be evaluated at  $t = t_{exp} = 0$  to get an estimate of the maximum values. The half-life of the compound in each compartment ( $t_{1/2,i}$ ) is equal to  $(\ln 2)/k_i$ . The first compartment is generally associated with the blood, the second with highly perfused tissues, the third with lesser perfused tissues, etc. The models were created by using GraphPad Prism (GraphPad Software, Inc., San Diego, CA), a nonlinear modeling program. Initial model input parameters were estimated using standard curve stripping procedures. Optimal models were selected based on minimization of standard error (SE) and 95% confidence intervals; appropriate

numbers of theoretical compartments were chosen based upon residuals analysis as described in the literature (20).

## Results and Discussion

**Data ranges.** Measurement of VOCs in a complex matrix such as blood is extremely difficult; the limiting factor for these experiments was the sensitivity of the blood analyses with a limit of quantitation (LOQ) of 100  $\mu\text{g/l}$ . The trichloroethene in breath was easily quantified below 1  $\mu\text{g/l}$ , although the experiment did not require that level of sensitivity. Overall, the relevant data range for blood samples was 100–1,600  $\mu\text{g/l}$  and <1–150  $\mu\text{g/l}$  for breath samples.

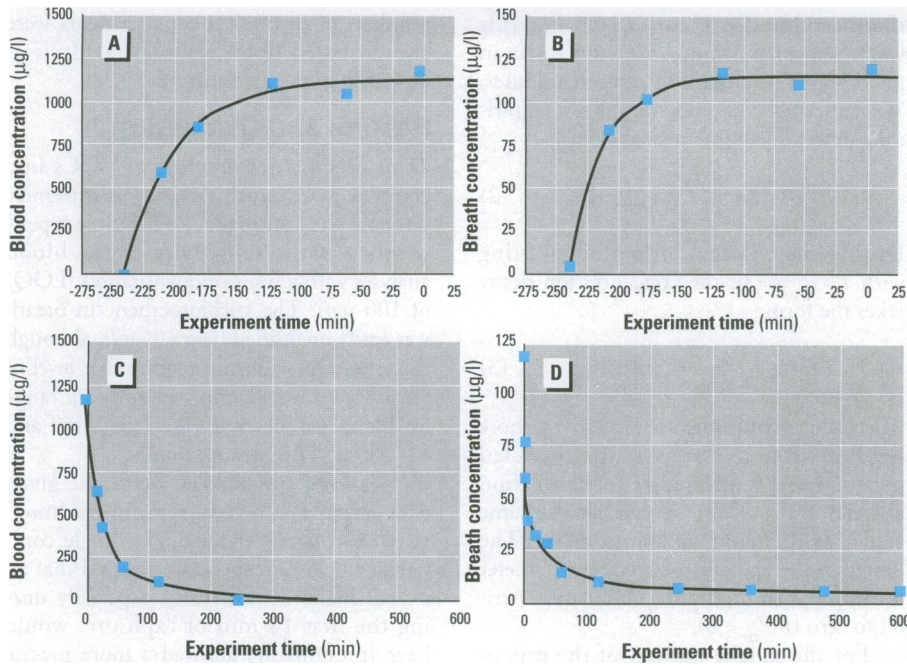
**Modeled parameters.** With the given data density and behavior, the uptake models were convergent for only a single compartment. In retrospect, we realized that an overall higher data density, especially during the first 15 min of exposure, would have (presumably) allowed a more precise multicompartmental model. Similarly, the elimination models for blood measurements were sufficient for only a two-compartment behavior, as the LOQ was quickly reached. The breath elimination curves were appropriately modeled with three compartments. Calculated model parameters for all trials are presented in Table 2. Regardless of the compartmental limitations, the modeling efforts were successful for comparing blood and breath data because the uptake curves tended to saturate during the 4-hr exposure period, and we had adequate data in the elimination phase to get excellent first compartmental fits for individual subjects. For illustration, we present a set of graphs of measurement and model for Subject 210 of uptake and elimination for both blood and breath in Figure 1A–D. Despite the individual differences among the six subjects, we see that, overall, the time dependence for inhalation uptake and that for elimination of trichloroethene are fairly similar from a cursory inspection of parameter means and SEs given in Table 2.

**Breath and blood time constants.** From the model parameters in Table 2 and inspection of Figure 1, we see that blood and breath measurements for trichloroethene are related and predictably covary. The exact shapes of the concentration versus time curves, however, demonstrate a definite trend wherein the blood response lags behind the breath measurements during periods of rapid change. During the uptake portion of the experiment, the time constant for breath is about 2.5 times as large as that for the blood, and during the initial 10 min of elimination (when the first compartment is dominant), the mean time constant for breath is 10 times as

**Table 2.** Modeled parameters for individual subjects

Matrix	Parameter	Subject 108	Subject 208	Subject 109	Subject 209	Subject 110	Subject 210	Mean $\pm$ SE
Uptake models								
Breath	$f^* C_{air} A_1$	105.8	98.59	130.3	147.1	116.8	113.0	118.6 $\pm$ 7.2
	$k_1$	0.07004	0.06985	0.07229	0.03236	0.01775	0.03660	0.04982 $\pm$ 0.00970
Blood	$f^* C_{air} A_1$	1,320	1,182	1,993	1,657	1,414	1,132	1,450 $\pm$ 133
	$k_1$	0.02606	0.02812	0.01068	0.01471	0.01518	0.02403	0.01980 $\pm$ 0.00293
Elimination models								
Breath	$A_1$	76.7	104.4	115.8	114.1	146.2	156.8	119.0 $\pm$ 11.8
	$k_1$	0.1576	0.7116	0.6052	0.2585	0.1704	0.6743	0.4296 $\pm$ 0.1066
	$A_2$	15.21	21.21	53.76	36.96	13.41	31.47	28.67 $\pm$ 6.26
	$k_2$	0.0128	0.02312	0.02552	0.03233	0.007861	0.0172	0.01981 $\pm$ 0.00365
	$A_3$	4.683	4.323	5.965	10.14	2.864	6.474	5.742 $\pm$ 1.022
	$k_3$	0.001631	0.001749	0.00128	0.002643	0.000546	0.000605	0.00141 $\pm$ 0.00032
Blood	$A_1$	820.7	899.5	1874	1403	501.8	918.5	1,069.6 $\pm$ 199.5
	$k_1$	0.02944	0.05548	0.03865	0.03188	0.1011	0.04505	0.0503 $\pm$ 0.0109
	$A_2$	349.4	385.1	287.0	238.0	979.1	264.4	417.2 $\pm$ 114.6
	$k_2$	0.002687	0.00455	0.002099	0.001313	0.01702	0.01065	0.00639 $\pm$ 0.00253

SE, standard error.



**Figure 1.** Illustration of a measured time course of trichloroethene exposure in Subject 210. (A) Uptake measured in blood (uptake curve model:  $C_{\text{blood}} = 1132 \{1 - e^{-0.02403(t+240)}\}$ ). (B) Uptake measured in breath model (uptake curve model:  $C_{\text{breath}} = 113 \{1 - e^{-0.0366(t+240)}\}$ ). (C) Elimination measured in blood model [elimination curve model:  $C_{\text{blood}} = 918 e^{-0.0450t} + 264 e^{-0.0107t}$ ]. (D) Elimination measured in breath model [ $C_{\text{breath}} = 157 e^{-0.6743t} + 31.5 e^{-0.0172t} + 6.47 e^{-0.00061t}$ ].

**Table 3.** Comparison of breath and blood data calculations for biological parameters

Parameter	Subject 108	Subject 208	Subject 109	Subject 209	Subject 110	Subject 210	Mean ± SE
<b>Uptake</b>							
Breath model value at $t = 240$ (µg/l)	105.8	98.6	130.3	147.0	115.2	113.0	118.3 ± 7.2
Blood - model value at $t = 240$ (µg/l)	1,317	1,181	1,839	1,608	1,377	1,128	1,408.6 ± 110.4
Breath, 1st compartment half-life (min)	9.90	9.92	9.59	21.42	39.05	18.94	18.1 ± 4.7
Blood, 1st compartment half-life (min)	26.60	24.65	64.90	47.12	45.66	28.85	39.6 ± 6.4
<b>Elimination</b>							
Breath model value at $t = 0$ (µg/l)	96.6	129.9	175.5	161.2	162.5	194.7	153.4 ± 14.3
Blood model value at $t = 0$ (µg/l)	1,170.1	1,284.6	2,161.0	1,641.0	1,480.9	1,182.9	1,486.8 ± 154.0
Breath, 1st compartment half-life (min)	4.40	0.97	1.15	2.68	4.07	1.03	2.4 ± 0.6
Blood, 1st compartment half-life (min)	23.54	12.49	17.93	21.74	6.86	15.39	16.3 ± 2.5
Breath, 2nd compartment half-life (min)	54.15	29.98	27.16	21.44	88.18	40.30	43.5 ± 10.1
Blood, 2nd compartment half-life (min) <sup>a</sup>	257.96	152.34	330.23	527.91	40.73	65.08	229.0 ± 75.0
Breath, 3rd compartment half-life (min)	424.98	396.31	541.52	262.26	1,269.50	1,145.89	673.4 ± 173.5
<b>Blood:breath ratios at end of exposure</b>							
Calculated from uptake models	12.45	11.97	14.12	10.94	11.96	9.99	11.91 ± 0.57
Calculated from elimination models	12.11	9.89	12.31	10.18	9.11	6.07	9.69 ± 0.93

SE, standard error.

<sup>a</sup>Values include contributions from unmodeled 3rd compartment.

large as its blood counterpart. For comparison of this timing issue, we have presented the compartmental half-lives for all subjects and experiments in Table 3. These constants are of great importance in eventually understanding the distribution of a contaminant among various target tissues. The differences between blood and breath time constants imply that there may be a technical issue in using breath measurement as a blood surrogate; however, the explanation could be that the breath is an excellent surrogate for the arterial blood, whereas the measurement is made in venous blood, which lags behind in concentration because of a finite mixing time in the body. Because the time constants for breath elimination are essentially identical to those found by Wallace et al. (21) at lower exposures, we feel confident that our interpretation of both blood and breath time constants is appropriate. This issue is explored in more detail in the section on blood/breath partition coefficients.

**Compartmental coefficients.** The linear coefficients of the models are representative of the relative amount an associated compartment contributes to the overall measured value, and their sum is proportional to the maximum concentration at equilibrium from a nondecreasing exposure. The fractional contribution from each compartment, of course, depends upon the exposure duration; a very short exposure (less than 10 min, for example) would not allow much transfer to the slower compartments, and essentially all of the contribution would thus be from the first compartment. For the work here, everything is based upon a 4-hr exposure that essentially equilibrates compartments 1, 2, and 3.

For the elimination phase, the quantity  $A_i / \sum A_i$  is the relative contribution of the  $i$ th compartment to the overall measurement. We calculated the first compartment contribution for all subjects as  $0.778 \pm 0.034$  (mean ± SE) and  $0.707 \pm 0.079$  for breath and blood measurements, respectively. This suggests that about 75% of the expired trichloroethene is coming from the blood compartment.

For the uptake phase, there were insufficient data to allow an accurate multicompartmental model; however, from Equation 1 we see that the evaluated composite parameter  $C(t = \infty) = f C_{\text{air}} \sum A_i$  can be used to estimate the expired:inspired concentration ratio  $f$  for trichloroethene as described in Equation 2 because we know  $\sum A_i = 1$  and  $C_{\text{air}}$  is the chamber concentration (100 ppmv or 541 µg/l at 23°C). For estimating this parameter based on blood data, we normalized by the appropriate average uptake partition coefficient of 11.91 as presented in Table 2. We found that the mean value

of  $f$  for all subjects using breath data was  $0.219 \pm 0.013$ , and using converted blood data it was  $0.225 \pm 0.021$ . These data show that the subjects metabolized, eliminated through nonexhalation routes, or stored about 78% of the inspired trichloroethene; that using blood or breath data gave equivalent results; and that the interindividual differences are small. Our value for  $f$ , the expired:inspired ratio measured at the high level exposure of 100 ppmv, is identical to the value found by Wallace et al. (21) for exposures at 10 ppbv.

**Peak blood levels.** Another biological parameter for which breath can be used as a surrogate for blood measurement is the estimation of peak blood level at the end of an exposure. Although an individual breath measurement at the end of the exposure (multiplied by an accepted blood/breath partition coefficient) could conceivably yield a very rough estimate, using the complete curves, and thus multiple data points, provides a more powerful estimate. Given the uptake and elimination equations as tabulated by parameter in Table 2, we evaluated all equations at  $t_{exp} = 0$ , the time at the end of the exposure from both directions; the results are given in Table 3. The blood equations yield fairly consistent estimated peak blood values, with respective means and SEs of the estimate:  $1,437 \pm 110$   $\mu\text{g/l}$  for uptake and  $1,487 \pm 154$   $\mu\text{g/l}$  for elimination. The breath estimates for peak levels have noticeable bias depending upon which set of equations is used:  $119 \pm 7.2$   $\mu\text{g/l}$  for uptake and  $153 \pm 14$   $\mu\text{g/l}$  for elimination. In using two-tailed paired  $t$ -tests, blood estimate means are not significantly different ( $p < 0.05$ ), whereas breath estimates are significantly different ( $p < 0.05$ ). This difference may be due to experimental factors; the elimination model depends greatly on a precisely defined time = 0 and upon the ability to collect simultaneous blood and breath samples, both of which are sometimes difficult to achieve logistically. Another potential source of difference may be that the  $fC_{air}$  term of Equation 1 is not actually a constant throughout rapid change. Certainly the concentration of trichloroethene in the blood just passing through the alveoli at time = 0 will be affected much more rapidly than in blood elsewhere in the body and thus strongly alter the associated breath levels. This would explain the stability of the blood-based estimates across the time = 0 boundary because blood samples are taken from a vein in the arm after some mixing in the body.

Under realistic conditions, we would have access to the subjects only during the elimination period because, presumably, they are busy working during an occupational

**Table 4.** Predicting blood levels from breath data (based on elimination curves of trichloroethene)

Subject I.D.	Breath ( $t = 0$ ) $\mu\text{g/l}$	Blood, calc		Blood, meas ( $\mu\text{g/l}$ )	Calc:meas ratio
		[breath $\times$ 9.2] ( $\mu\text{g/l}$ )			
108	96.6	889		1,170	0.76
208	129.9	1,195		1,285	0.93
109	175.5	1,615		2,161	0.75
209	161.2	1,483		1,641	0.90
110	162.5	1,495		1,481	1.01
210	194.7	1,792		1,183	1.51
Mean $\pm$ SE		1,411 $\pm$ 131.4		1,487 $\pm$ 154.05	0.98 $\pm$ 0.12

Abbreviations: calc, calculated; meas, measured; SE, standard error.

exposure; therefore, we chose to use the elimination period as the relevant data set. From the literature, we find values for the blood/air partition coefficient of 8.11 from Gargas et al. (22), 15.7 from Lindqvist (23), and 8.1 from Fiserova-Bergerova and Diaz (24), all measured *in vitro*. Additionally, Fiserova-Bergerova and Diaz (24) present 14.0 as the measured *in vitro* partition coefficient for lung tissue, and Fernandez et al. (25) use 9 as the pulmonary partition coefficient. These values lead us to believe that the blood/breath coefficient at equilibrium is most likely bounded on the low side by 8.1 and on the high side by 16. Allen and Fisher (26) have developed a successful pharmacokinetic model for trichloroethene in humans wherein they use the value 9.2 as gleaned from various sources. Accordingly, we chose 9.2 as the accepted literature value. We use the term blood/breath partition coefficient in the sense of Gargas et al. (22) and others to mean the ratio of the measured blood concentration to the measured breath concentration; we do not presume to distinguish between venous and arterial blood or to present a more theoretical approach involving cardiac output and alveolar ventilation rate.

For each subject, we predicted the peak blood level from the elimination breath data by using the value 9.2 as the blood/breath coefficient and compared these results to the actual blood value as derived from the elimination period blood measurements. Table 4 presents the respective values, differences, and statistics. The average value of the predicted:measured is  $0.98 \pm 0.12$  (SE). A two-tailed paired  $t$ -test shows that the means are not significantly different for  $p < 0.05$ . Thus we see that blood peak levels can be well predicted from breath data and, again, that individual differences between individuals have a relatively small effect.

**Calculated blood/breath partition coefficients.** Although there is appreciable scatter in all of our empirical data, we found that for the time near the break between exposure and elimination periods when the venous and arterial blood are essentially in equilibrium, we could predict blood values from breath measurements using an accepted literature

value for the blood/breath partition coefficient. Using the empirically based model equations for each individual, we calculated the apparent blood/breath partition coefficient at the conclusion of the 4-hr exposure for both the uptake and elimination period data. This is the most crucial time in a non-decreasing exposure because it reflects both the highest concentrations and the most stable (closest to equilibrium) portion of the experiment. The data for each individual's partition coefficients at this critical time are given in Table 3 for all data sets. Our calculated partition coefficient at experiment time = 0 for all trials ranges from 6 to 14, with an overall mean of 10.8, which is in essential agreement with the range of literature values. Using the elimination only data set, the mean  $\pm$  SE =  $9.69 \pm 0.93$ .

When calculated for different instances during the experiment, the blood/breath ratio is reasonably consistent during the uptake period; however, it becomes erratic and appreciably higher during the elimination period. This puzzling behavior is illustrated in Figure 2, where we have plotted series of data points synthetically generated from the models for all subjects. Instability in the partition coefficient during changing concentrations in the elimination period has been noted by Wallace et al. (21) in their study of various VOCs and by Buckley et al. (27) in their study of MTBE exposure, where they attribute this behavior to a hypothetical mucous compartment. In our case, we suspect that we are experiencing a timing difference; the breath is reflecting arterial blood, whereas the blood measurement is being made from the slower changing venous blood. We expect that the venous blood would have a higher concentration than the arterial blood during elimination because, as the fastest compartment, it collects trichloroethene from the slower tissue groups and delivers it to the lungs. Therefore, in the nonequilibrium elimination phase, we would expect the partition coefficient to increase, at least temporarily. Another consideration is that the LOQ for blood measurements did not allow a strong modeling effort for a third

compartment, and therefore the elimination phase models suffered somewhat in describing the behavior. Finally, intersubject differences may also be driven by competitive elimination process rates.

The intersubject differences in the measured blood/breath ratio during the elimination cannot be attributed to the physiognomy of subjects or to the relatively limited sensitivity of the blood measurement. The

two curves in Figure 2 exhibiting a significant decrease are from Subjects 110 and 210; one is a 65 in tall, 139-lb female and the other a 70 in tall, 134-lb male. Secondly, study of blood/breath ratio versus blood concentration separately for the uptake and elimination periods indicates that there is a definite trend towards greater variability and higher absolute value for the elimination period. Figure 3 presents the scatter plots

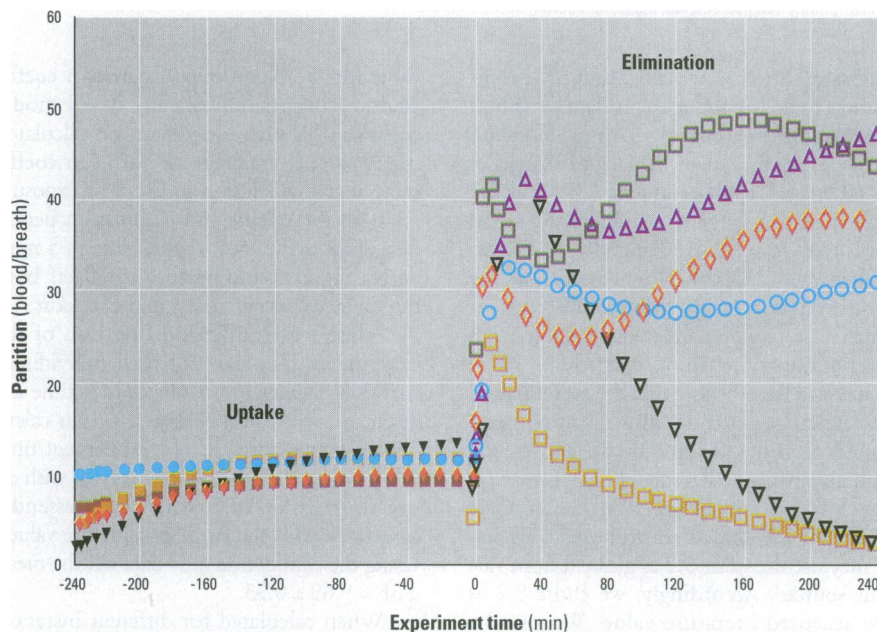
and regression lines for that region where the blood concentrations overlap.

**Blood versus breath data relationship.** The overall blood versus breath relationship for all individual data point pairs is graphed as simple linear regressions in Figure 4A and B for the uptake and elimination phases for illustration. We note that the blood/breath regression slope for uptake is appreciably different from that for elimination (10.93 vs. 29.00, respectively). Although the data exhibit appreciable scatter, the slopes are significantly nonzero ( $p < 0.0001$ ). Similar regressions for individual subjects exhibit a tighter linear relationship, which suggests that there are definite differences between test subjects at times of changing concentration. However, the qualitative behavior of the venous blood and exhaled breath measurements, as illustrated in Figure 1, is very similar and the blood/breath partition coefficients extracted by using modeled curves evaluated at  $t_{exp} = 0$  in Table 3 are comparable. Therefore, use of a blood/breath partition coefficient based upon one blood/breath data pair during a time of concentration change may be risky without knowing more of the previous time history of the exposure.

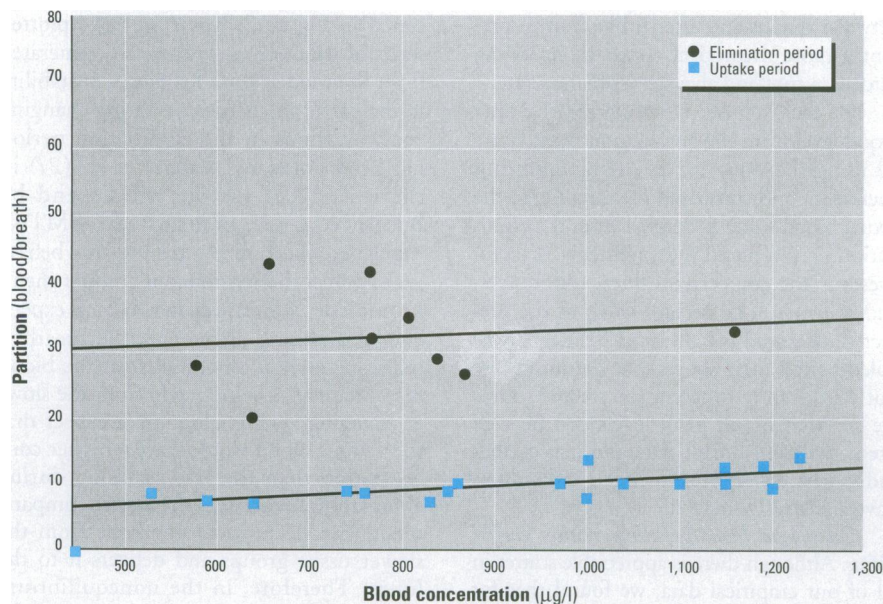
## Conclusions and Recommendations

Breath measurements are an excellent surrogate for determining the qualitative behavior of blood concentrations during and after inhalation exposure to trichloroethene, even under rapidly changing conditions. Both blood and breath time-series measurements can be mathematically described by simple multiexponential uptake and elimination models; interpretation of these models yields information about compartmental residence time of a pollutant in the body and the relative capacity for compartments. We conclude that at equilibrium our subjects excrete only about 22% of their inspired trichloroethene through exhalation and that the first compartment (blood) contributes about 75% of that amount. Therefore, about 78% of all trichloroethene that enters the body during inhalation exposure is metabolized, stored, or excreted through routes other than exhalation and 66% of this activity occurs in the deeper compartments.

The use of modeled empirical data and published blood/breath partition coefficients has been demonstrated to accurately predict peak blood levels from breath data after a nondecreasing exposure. This is an important pragmatic conclusion for exposure assessment because the noninvasive breath measurement after an exposure becomes a surrogate for the invasive blood



**Figure 2.** Illustration of the pattern of the blood/breath partition coefficient as calculated for all subjects from the respective uptake and elimination models. During the uptake phase, when experiment time is  $< 0$ , the calculated partition coefficient is relatively stable; during the elimination phase, the measured coefficient becomes erratic.



**Figure 3.** Comparison of relative values of the blood/breath partition coefficient for the uptake and the elimination periods with respect to measured blood concentration in the range where these periods overlap. Anomalous behavior of partition coefficients cannot be attributed to a blood measurement problem. Regression lines indicate a slightly positive slope.

measurement during the exposure. The results are most likely valid even for moderate activity during a prolonged exposure as long as the subject is at rest during the subsequent elimination period when the samples are taken. We conclude that the blood/breath partition coefficient value that we measured as the average value for all subjects and for both uptake and elimination phase extrapolations (10.8) is as accurate a value for high-level inhalation exposure for trichloroethene as is currently available. For predicting venous blood peak values from elimination only breath data, the appropriate coefficient is 9.69 to allow for blood mixing in the body.

Quantitative differences were found for measuring tissue compartment half-lives using blood and breath data. Changes in concentration occurred faster in breath than blood, typically by a factor of 10. We conclude that this is primarily attributable to the timing difference between the direct venous blood measurement and the breath measurement, which is more related to the arterial blood. Also, we found that the elimination phase blood/breath ratios were appreciably greater than those during the uptake phase, with the uptake value close to various published values. We conclude that this is due, in part, to the difference between venous and arterial blood, the experimental logistics of taking simultaneous samples, and the limiting factor of sensitivity in the blood measurement.

The anomalous behavior of the blood/breath ratios during the elimination period, as shown in Figure 2, appears to be a real phenomenon. This is confirmed by the data shown in Figure 3, which shows no statistically significant concentration dependent trends in the overlap region with the uptake period. Also, the physical characteristics of the subjects are not apparently relevant.

Though this is admittedly based upon a small subject set, we conclude that individual measurements of blood/breath during the elimination period are highly variable among subjects for reasons most likely involving differences in relative elimination rates from breath with respect to other routes such as metabolism and storage.

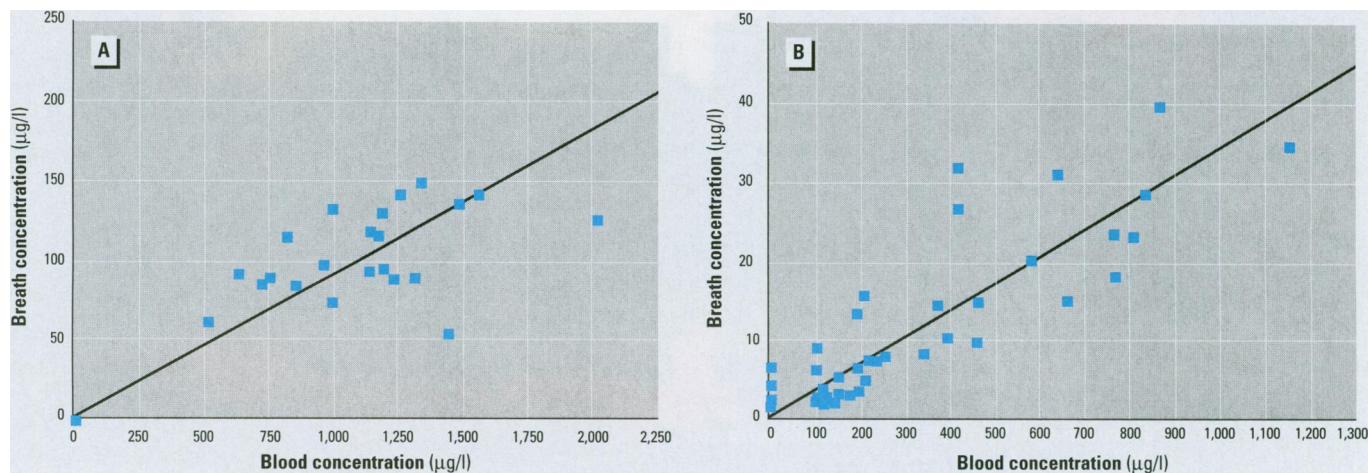
This empirical measurement work points out some areas for future study. The relationships among venous blood, exhaled breath, and arterial blood concentrations of a distributed pollutant should be further investigated with refined experimental methods to eliminate as much measurement uncertainty as possible; in particular, the sensitivity of the blood measurement should be improved to allow for higher compartmental modeling. The information presented here should be considered in future pharmacokinetic modeling efforts, especially in regard to the unanticipated, yet fairly consistent jump in blood/breath ratio from 10 to 30 across the uptake to elimination boundary. Specifically, we recommend investigation into the effects of different levels of exposure, higher frequency sampling during the initial uptake and initial elimination periods, and a very detailed look at differences between individuals based upon physiological parameters. The following are critical questions that need to be answered to allow a better understanding of the internal biological processes from trichloroethene exposure: What does an empirically determined blood/breath partition coefficient actually mean when concentrations are changing rapidly? Why are there large differences between respective breath and blood time constants? Answers to these questions will require more measurements combined with a specific mathematical modeling effort that incorporates the empirical data. We

concur with the philosophy of Blancato (28) that

... modeling and models do not replace well planned and precisely performed laboratory experiments. Rather, they are adjuncts which serve to maximize usefulness of experimental results, assist in the more precise planning of other meaningful experiments, and help design cost effective and meaningful field monitoring studies.

## REFERENCES AND NOTES

- Mason MF, Dubowski KM. Breath alcohol analysis: uses, methods, and some forensic problems. *J Forensic Sci* 21:9-41 (1976).
- Wallace LA, Pellizzari ED. Recent advances in measuring exhaled breath and estimating exposure and body burden for volatile organic compounds (VOCs). *Environ Health Perspect* 103(suppl 3):95-98 (1996).
- Wallace LA, Buckley TJ, Pellizzari ED, Gordon SM. Breath measurements as VOC biomarkers: EPA's experience in field and chamber studies. *Environ Health Perspect* 104(suppl 5):861-869 (1996).
- Phillips M. Breath tests in medicine. *Sci Am* (July):74-79 (1992).
- Chinery RL, Gleason AK. A compartmental model for the prediction of breath concentration and absorbed dose of chloroform after exposure while showering. *Risk Anal* 13:51-62 (1993).
- Weisel CP, Jo WK, Liou PJ. Utilization of breath analysis for exposure and dose estimates of chloroform. *J Expo Anal Environ Epidemiol*(suppl 1):55-69 (1992).
- Raymer JH, Pellizzari ED, Thomas KW, Cooper SD. Elimination of volatile organic compounds in breath after exposure to occupational and environmental microenvironments. *J Expo Anal Environ Epidemiol* 1:439-451 (1991).
- Wallace LA, Pellizzari ED, Gordon SM. A linear model relating breath concentrations to environmental exposures: application to a chamber study of four volunteers exposed to volatile organic chemicals. *J Expo Anal Environ Epidemiol* 3:75-102 (1993).
- McKone TF. Linking a PBPK model for chloroform with measured breath concentrations in showers: implications for dermal exposures. *J Expo Anal Environ Epidemiol* 3:339-365 (1993).
- Andersen ME. A physiologically based toxicokinetic description of the metabolism of inhaled gases and vapors: analysis at steady state. *Toxicol Appl Pharmacol* 60:509-526 (1981).
- Aggazzotti G, Fantuzzi G, Predieri G, Righi E, Moscardelli S. Indoor exposure to perchloroethylene



**Figure 4.** Scatter plot and linear regression of individual measurements plotted as breath versus blood for all subjects. (A) Data from uptake phase shows a mean blood/breath ratio of 10.93. (B) Data from elimination phase shows a mean blood/breath ratio of 29.00.

- (PCE) in individuals living with dry-cleaning workers. *Sci Total Environ* 156:133-137 (1994).
12. Pleil JD, Lindstrom AB. Collection of a single alveolar exhaled breath for volatile organic compounds analysis. *Am J Ind Med* 28:109-121 (1995).
  13. Pleil JD, Lindstrom AB. Measurement of volatile organic compounds in exhaled breath as collected in evacuated electropolished canisters. *J Chromatogr B: Biomed Appl* 665:271-279 (1995).
  14. Lindstrom AB, Pleil JD. Alveolar breath sampling and analysis to assess exposures to methyl tertiary butyl ether (MTBE) during motor vehicle refueling. *J Air Waste Manage Assoc* 46:676-682 (1996).
  15. Lindstrom AB, Pleil JD. A methodological approach for exposure assessment studies in residences using volatile organic compound contaminated water. *J Air Waste Manage Assoc* 46:1058-1066 (1996).
  16. Pleil JD, Lindstrom AB. Exhaled human breath measurement for assessing exposure to halogenated volatile organic compounds. *Clin Chem* 43(5):723-730 (1996).
  17. Lindstrom AB, Pleil JD, Berkoff DC. Alveolar breath sampling and analysis to assess trihalomethane exposures during competitive swimming training. *Environ Health Perspect* 105:636-642 (1997).
  18. Bishop CT, Brashear WT, Pollard DL. Analysis of Trichloroethylene: Standard Operating Procedure. SOP No. 4300-1063. Dayton, OH:ManTech Environmental Technology, Inc., 1995.
  19. Winberry WT, Murphy NT, Riggan RM. Method T0-14. In: *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air*. EPA-600/4-89-017. Research Triangle Park, NC:U.S. Environmental Protection Agency, 1989.
  20. Pleil JD, Lindstrom AB. Sample timing and mathematical considerations for modeling breath elimination of volatile organic compounds. *Risk Anal* (in press).
  21. Wallace LA, Nelson WC, Pellizzari ED, Raymer JH. Uptake and decay of volatile organic compounds at environmental concentrations: application of a four-compartment model to a chamber study of five human subjects. *J Expo Anal Environ Epidemiol* 7:141-163 (1997).
  22. Gargas ML, Burgess RJ, Voisard DE, Cason GH, Andersen ME. Partition coefficients of low molecular weight volatile chemicals in various liquids and tissues. *Toxicol Appl Pharmacol* 98:87-99 (1989).
  23. Lindquist T. Partition coefficients of blood/air and water/air for some commonly used solvents. In: *Industrial and Environmental Xenobiotics: In Vitro Versus In Vivo Biotransformation and Toxicity* (Fouts JR, Gut I, eds). Amsterdam:Excerpta Medica, 1978.
  24. Fiserova-Bergerova V, Diaz M. Determination and prediction of tissue-gas partition coefficients. *Int Arch Occup Environ Health* 58:75-87 (1986).
  25. Fernandez JG, Droz PO, Humbert BE, Caperos JR. Trichloroethylene exposure: simulation of uptake, excretion, and metabolism. *Br J Ind Med* 34:43-55 (1977).
  26. Allen BC, Fisher JW. Pharmacokinetic modeling of trichloroethylene and trichloroacetic acid in humans. *Risk Anal* 13(1):71-86 (1993).
  27. Buckley TJ, Prah JD, Ashley D, Zweidinger RA, Wallace LA. Body burden measurements and models to assess inhalation exposure to methyl tertiary butyl ether (MTBE). *J Air Waste Manage Assoc* 47:739-752 (1997).
  28. Blancato JN. Pharmacokinetics, chemical interactions, and toxicological risk assessment in perspective. *Environ Health Perspect* 102(suppl 9):133-137 (1994).

## NIEHS Excellence in basic research at the National Institute of Environmental Health Sciences

NIEHS scientists and grantees are performing basic studies of our susceptibility to environment-related disease: demonstrating that a carcinogen in cigarette smoke (benzo(a)pyrene) alters part of a gene to cause lung cancer . . . showing the effects of fetal exposure to PCBs . . . developing a strain of mouse that lacks functional estrogen receptors and that helps evaluate how some pesticides and other estrogen-like compounds might affect development and reproduction . . . discovering the genes for breast, ovarian, and prostate cancers . . . identifying women's optimal days of fertility . . . seeking to reverse the damage from lead exposure . . . finding alternatives to traditional animal tests . . . pinpointing the functions of specific genes by eliminating them from specially bred mouse lines . . . discovering a way, using ordinary yeast cells, to isolate and clone genes and other fragments of genetic material more quickly . . . showing the effects of urban air on lung function . . .

A part of the National Institutes of Health, the National Institute of Environmental Health Sciences is located in Research Triangle Park, North Carolina.

See the NIEHS home page: <http://www.niehs.nih.gov>