Effect of Water Temperature on Dermal Exposure to Chloroform

Sydney M. Gordon,1 Lance A. Wallace,2 Patrick J. Callahan,1 Donald V. Kenny,1 and Marielle C. Brinkman1

1Battelle Memorial Institute, Columbus, OH 43201 USA; 2National Exposure Research Laboratory, U.S. Environmental Protection Agency, Reston, VA 20192 USA

We have developed and applied a new measurement methodology to investigate dermal absorption of chloroform while bathing. Ten subjects bathed in chlorinated water while breathing pure air through a face mask. Their exhaled breath was delivered to a glow discharge source/ion trap mass spectrometer for continuous real-time measurement of chloroform in the breath. This new method provides abundant data compared to previous discrete time-integrated breath sampling methods. The method is particularly well suited to studying dermal exposure because the full face mask eliminates exposure to contaminated air. Seven of the 10 subjects bathed in water at two or three different temperatures between 30°C and 40°C. Subjects at the highest temperatures exhaled about 30 times more chloroform than the same subjects at the lowest temperatures. This probably results from a decline in blood flow to the skin at the lower temperatures as the body seeks to conserve heat forcing the chloroform to diffuse over a much greater path length before encountering the blood. These results suggest that pharmacokinetic models need to employ temperature-dependent parameters. Two existing models predict quite different times of about 12 min and 29 min for chloroform flux through the stratum corneum to reach equilibrium. At 40°C, the time for the flux to reach a near steady-state value is 6-9 min. Although uptake and decay processes involve several body compartments, the complicating effect of the stratum corneum lag time made it difficult to fit multicompartmental models to the data; however, a single-compartment model gave a satisfactory fit. Key words: breath, chloroform, dermal exposure, drinking water, pharmacokinetics, real time, residence times. Environ Health Perspect 106:337–345 (1998). [Online 13 May 1998]
http://ehpnet1.niehs.nih.gov/docs/1998/106p337-345gordon/abstract.html

Chloroform in water is a by-product of the chlorination process (1). Bathing or showering in chlorinated tap water exposes individuals to chloroform by ingestion, inhalation, or dermal contact. Some epidemiological studies have suggested that exposure to chlorinated water causes bladder cancer (2–4) and is associated with rectal cancer (4) and potential birth defects (5).

Several studies, including some based on exhaled breath analysis, suggest that significant dermal exposure to chloroform occurs while showering, and the dose is roughly comparable to that resulting from inhalation (6–9). More recent studies have extended this work to swimming in indoor pools (10–14). Most of these investigations have used breath measurements to determine total exposure. Because of the dynamic equilibrium between the concentration of a volatile organic compounds (VOCs) in the blood and their concentration in exhaled breath (15), breath measurements can be used to estimate body burden and to detect changes in body burden with time (16–19).

An important question concerns the relative contribution of dermal exposure to total exposure during showers and baths. It has been difficult to answer this question because there has not been a way to isolate each of the two pathways (inhalation and dermal absorption). Previous studies have measured breath levels due to combined dermal and inhalation exposure or inhalation exposure only, using a subtraction method to estimate the dermal contribution (6,13). We have developed a method to measure dermal absorption only, as described below, and applied the method to bathing in domestic tap water.

Most previous measurements of human breath concentrations of chloroform to determine the dose resulting from dermal or inhalation exposure to tap water have relied on the use of integrated sampling methods and subsequent batch analysis. This has limited the number of samples that are typically collected in any exposure study to about four during the uptake phase and usually no more than about 12 during the decay phase, thus reducing the reliability of data designed to address these issues.

We have developed new monitoring technology to obtain better time resolution over the uptake and elimination periods of an exposure episode (18,20). The technology, which can measure trace VOCs in air or exhaled breath continuously in real time, consists of a direct sampling system (glow discharge ionization source) and a compact ion trap mass spectrometer (ITMS) that is capable of operation in the full tandem (MS/MS) mass spectrometric mode (21–23). For breath analysis, a specially designed inlet system is attached to the glow discharge source and provides a constant flow of exhaled air (18,20). This direct sampling and analysis approach offers a powerful means of extracting VOCs directly from the breath matrix and eliminates the preconcentration step that normally precedes exhaled air analysis by conventional gas chromatography/mass spectrometry (GC/MS) (17,24).

Preliminary work with this equipment included continuous breath measurements of one person in an outdoor swimming pool, as well as measurements of four persons taking hot baths indoors. The person in the outdoor swimming pool received almost no exposure through dermal absorption, although chloroform levels in the water were 70 µg/l. We therefore had one subject bathe in cool water (30°C) and then in hot water (40°C). Breath levels hardly changed from preexposure levels during the time in the cool water, but increased markedly after several minutes at the higher temperature. These preliminary observations led us to hypothesize that dermal absorption is quite limited in water that is cooler than some value between 30 and 40°C; therefore, we have carried out a set of experiments to test this hypothesis. Six additional subjects took baths in chlorinated water at various temperatures and their breath levels of chloroform were continuously monitored.

Methods

Exposure Conditions and Breath Sampling Protocol

The experiments were carried out in two stages. In the first stage, we used four volunteers to determine the rate of diffusion of chloroform through the skin by immersing...
them in a bath containing domestic tap water at a fixed temperature and measuring the resulting breath chloroform levels in real time while isolating them from the inhalation component of the exposure. One of the subjects was also exposed at two different water temperatures. The second stage made use of six volunteers, each of whom provided breath samples during and after dermal exposure to chloroform at three water temperatures. Each subject was fitted with a facemask (Model 8932; Hans Rudolph, Inc., Kansas City, MO), equipped with a two-way nonbreathing valve set, which was attached to a purified air supply to ensure that there was no exposure to headspace chloroform above the bathtub. Each subject inhaled clean air through the first one-way valve and exhaled through the second valve into the mixing chamber of the breath inlet system. The fit of the mask was checked for air infiltration before each experiment by comparing the signal response with that obtained with zero-grade air. Information on the subjects and the conditions in each experiment are provided in Table 1. The study protocol was reviewed and approved by the Battelle Human Subjects Committee, and informed consent was obtained from each subject.

The subjects were instructed to drink only bottled water beginning the evening before the experiment and to refrain from drinking any bottled beverages, bathing or showering the morning of the experiment, and using perfumes or after-shave lotions. Water samples were collected into clean glass vials with Teflon-lined caps and analyzed using standard procedures to determine the concentrations of chloroform in the water (25). Because the subjects did not undergo inhalation exposure during the course of the experiments, no air samples were collected for analysis.

**First stage.** For the experiments in the first stage, a standard bathtub in a bathroom was filled with tap water and the temperature adjusted to about 40°C. Before entering the bathroom, the subject changed into a swimsuit and put on the face mask. The outlet tube from the face mask was attached to the real-time breath analyzer when the subject was in the bathroom and immediately before he/she stepped into the tub. Upon providing a preexposure breath sample, the subject climbed into the bathtub and immersed him/herself in the water up to shoulder height. Chloroform breath measurements were made every 6 sec while the subject continued to breathe purified air. The water temperature was maintained at about 40°C with a thermocouple gauge, which was read manually; hot water from the bathtub faucet was added as needed. After 20–25 min, when the real-time breath analyzer indicated that the breath chloroform level had reached a plateau, the subject stepped out of the bathtub and quickly dried him/herself while continuing to breathe purified air and exhale into the analyzer. Postexposure breath measurements were taken for about 30 min before the subject was allowed to remove the face mask and rest in a nearby room. After about 10 min, another brief set of breath measurements were taken for 5–8 min to confirm that the breath chloroform level was at, or close to, the original preexposure level.

One of the subjects (subject 4) participated in a separate experiment that explored the effect of water temperature on the absorption. The water temperature was initially adjusted to about 30°C, and breath measurements were taken continuously for about 15 min. Then, the temperature was raised to about 40°C and breath measurements continued for a further 27 min until the concentration of chloroform in the breath began to level off.

**Second stage.** The second set of experiments was carried out in a 380-liter stainless steel hydrotherapy tub (109 cm long × 53 cm wide × 71 cm high). The tub was connected to a domestic hot and cold water supply and, immediately before the start of an experiment, the water inflow was adjusted to give the desired temperature, as indicated by a digital thermometer (Model 52; Fluke Corporation, Everett, WA). A water sample was collected while the subject provided a preexposure breath sample; the subject then stepped into the tub and immersed him/herself in the water up to neck height. Chloroform breath measurements were made every 12 sec while the subject continued to breathe purified air; readings of the water temperature were taken manually at regular intervals throughout the exposure period. Figure 1 is a schematic of the subject in the filled hydrotherapy tub; the subject wears the face mask, which is attached to the pure air supply, and exhales into the breath analyzer. Measurements at the two lower temperatures (30 and 35°C) were taken until the breath chloroform concentrations in each case appeared to level off before the subject stepped out of the tub. As before, at the higher temperature (40°C), measurements were

| Subject | Sex | Height (cm) | Weight (kg) | Age (year) | Chloroform concentration in water (µg/l) | Water temperature (°C) | Exposure time (min) |
|---------|-----|-------------|-------------|-----------|---------------------------------|----------------------|------------------|
| 1       | M   | 173         | 81.6        | 35        | 91                               | 41                   | 24.4             |
| 2       | F   | 180         | 72.6        | 32        | 91                               | 40                   | 29.9             |
| 3       | M   | 168         | 56.7        | 26        | 91                               | 40                   | 29.9             |
| 4       | F   | 168         | 52.2        | 26        | 81                               | 40                   | 27.5             |
| 5       | M   | 168         | 56.7        | 26        | 54.5/78/39.5                      | 32.4/36.0/39.7       | 25.4/32.2/1*     |
| 6       | F   | 157         | 65.8        | 38        | 86.5/90.5/92.5                    | 32.1/35.0/39.6       | 30/30/30         |
| 7       | M   | 183         | 79.4        | 23        | 89.5/94.5/98                      | 28.2/34.3/38.8       | 30/29.8/29.2     |
| 8       | F   | 170         | 70.3        | 39        | 88/93.5/98                        | 28.8/33.8/38.3       | 29.2/29.8/29.8   |
| 9       | F   | 160         | 61.2        | 27        | 10/1/98                          | 29.5/34.8/38.3       | 30/30/29.2       |
| 10      | M   | 165         | 102         | 23        | -92.5/93                         | -56.7/93.8           | -29.8/29.8       |

Abbreviations: M, male; F, female.

*A/B/C where A = value for the low temperature experiment; B = value for the medium temperature experiment; and C = value for the high temperature experiment.

![Figure 1. System for sampling exhaled breath samples in real time from a subject exposed by dermal absorption to pollutants in water.](image-url)
taken from the start of the exposure until a plateau was reached, at which time the subject climbed out of the tub and continued to exhale into the analyzer while breathing purified air. Postexposure breath measurements were again taken for about 30 min before the subject removed the face mask. A second water sample was collected immediately after the subject stepped out of the tub.

**Breath Analysis**

The real-time breath analyzer consists of the breath inlet unit, the direct breath sampling interface, and the ITMS. Figure 2 shows the breath inlet attached to the analytical system (18,20). The mouthpiece and two one-way valves normally used with this unit were replaced for the work described here with the Rudolph Model 8932 face mask (Hans Rudolph, Inc.), which contains a two-way nonbreathing valve set, to isolate the subject from the chloroform in the air. Purified air for breathing was supplied by a gas cylinder, and the air was then fed via a large-diameter Teflon tube into a 1.3-liter glass mixing chamber. The undiluted breath sample is drawn at a constant rate from the mixing chamber by the vacuum in the glow discharge source and flows into the ionizer at a rate of about 190 ml/min without any attention from the subject.

The direct breath sampling interface (Fig. 2) is a glow discharge ionization source, which is attached to the ITMS. The operation of this system has been described in detail elsewhere (26–28). For the work described here, we used a Teledyne Electronic Technologies (Mountain View, CA) 3DQ Discovery ITMS as the analyzer (29). The 3DQ is a compact, field-deployable instrument with high sensitivity and specificity (21–23). Ions at m/z 83 and 47 were selected as the parent and product ions for chloroform, respectively. However, low ion intensity and limited MS/MS conversion efficiency (25%) in the first set of experiments prevented any monitoring for chloroform in the MS/MS mode. Instead, we were forced to use only the molecular ion at m/z 83. Because of a small contribution present in an interferent, there was a slightly higher background level than would be expected for breath chloroform under normal conditions (30). Before undertaking the second set of experiments, the ITMS was modified to reduce the level of background noise from the glow discharge source (31), and the improved signal-to-noise ratio allowed us to measure chloroform in the MS/MS mode at m/z 47. This effectively eliminated the problem due to the interferent that was observed in the initial experiments.

To calibrate the real-time breath analyzer in the laboratory, chloroform gas standards were prepared in a 186-liter glass chamber.

Pure chloroform (0.2 μl) was injected via a septum into humidified ultra-high purity air in the chamber at room temperature. A stainless steel fan in the chamber blended the standard with the air. Humidified ultra-high purity air was added to the chamber to dilute the chloroform concentration to about 100 μg/m³ (21 ppb)—a level slightly above the maximum breath concentrations expected in the exposure measurements. Then, 18-liter and 6-liter samples were taken from each of the chambers in Tedlar bags (SKC Inc., Eighty Four, PA) and evacuated stainless steel canisters, respectively. The two canister samples were analyzed by a modified EPA method, TO-14 (32). The concentrations measured in these analyses were assumed to apply to the samples in the two Tedlar bags that were collected at the same time as the canister samples.

To calibrate the breath analyzer for the exposure experiments in the field, each Tedlar bag sample was connected to the sample line at the point where it attached to the breath inlet unit. In this way, the calibration gas standards flowed through the same length of tubing as the actual breath samples. The ITMS was used to isolate the parent or product mass, and the resultant ion signals were measured at the two concentrations. The Tedlar bag samples were used to recalibrate the instrument immediately before and after breath samples were taken from each subject.

**Data Analysis Methods**

**Percutaneous permeation.** Qualitatively, the breath concentration immediately after submersion in the bathtub should follow an S-shaped curve (Fig. 3) with 1) an initial period T₀ of no change from background as the chloroform diffuses through the skin; 2) a period T₁ when the concentration increases in an accelerated way—the slope goes from zero to a maximum value; and 3) a final period when the concentration continues to increase but slows to approach an asymptote—the slope goes from a maximum value back to zero.

The measured breath uptake curves may be used to examine the three portions of the S-shaped curve. The first period (T₀) lasts from the time the subject is immersed to the time of the first measurable increase in the breath concentration. We determined the value of T₀ by calculating the standard deviation S of the background breath values and requiring a 3S increase over background.

Pirot et al. (33) have modeled the stratum corneum (SC) as a homogeneous flat plate satisfying the diffusion equation everywhere:

\[
\frac{\partial C(x,t)}{\partial t} = -D \frac{\partial^2 C}{\partial x^2},
\]

(1)
where \( D \) is the diffusion coefficient and \( x \) measures the depth of penetration into the SC. From the theory of heat conduction in solids, an exact solution to the diffusion equation for an infinite plate of thickness \( L \), where the two sides of the plate are held at two constant temperatures, has been given by Carslaw and Jaeger (34). Replacing temperature with chloroform concentration and setting the concentration equal to zero at the inner edge of the SC (because of the rapid loss of chloroform as the blood in the capillaries removes it from the skin), the concentration \( C(x,t) \) everywhere in the SC during exposure to a constant chloroform concentration in the water \( C_{\text{water}} \) is

\[
C(x,t) = \frac{2P_{\text{sw}}C_{\text{water}}}{\pi L} \sum_{n=1}^{\infty} \frac{(-1)^{n-1}}{n} \sin\left(\frac{n\pi x}{L}\right) e^{-Dn^2\pi^2t}/L^2.
\]

(2)

Here, \( P_{\text{sw}} \) is the skin–water partition coefficient. The terms involving time \( t \) in Equation 2 disappear finally, leaving an equilibrium concentration in the SC described by a straight line sloping downward from a value of \( P_{\text{sw}}C_{\text{water}} \) at the outer edge of the SC (\( x = L \)) to a value of zero at the inner edge (\( x = 0 \)).

The flux across any point \( x (0<x<L) \) in the SC is

\[
-D \frac{\partial C}{\partial x} |_{x=L} = D P_{\text{sw}} C_{\text{water}}
\]

and the flux into the body (at \( x = L \)) during constant dermal exposure is

\[
+2DP_{\text{sw}} C_{\text{water}} \sum_{n=1}^{\infty} \cos\left(\frac{n\pi x}{L}\right) e^{-Dn^2\pi^2t}/L^2.
\]

(4)

Finally, assuming that the exposure suddenly drops to zero (as the subject leaves the tub) and that he/she has attained equilibrium while in the tub, the solution for the concentration in the SC is given by

\[
C(x,t) = \frac{2P_{\text{sw}}C_{\text{water}}}{\pi L} \sum_{n=1}^{\infty} \frac{1}{n} (-1)^{n-1} \frac{Dn^2\pi^2}{L^2} \sin\left(\frac{n\pi x}{L}\right) e^{-Dn^2\pi^2t}/L^2.
\]

(5)

where \( t \) is measured from the time exposure ends. Differentiation of this expression as in Equation 3 and evaluation at \( x = 0 \) and \( x = L \) provides the flux into and out of the body after exposure ends. Because more of the chloroform is contained in the outer half of the SC, more diffuses outward than into the body; in fact, about two-thirds of the chloroform in the SC diffuses outward after the end of exposure.

**Linear compartmental model.** Although the theory outlined above provides elegant solutions for the concentration in and flux across the SC as a function of the water concentration, we must still make a connection between the measured quantity (exhaled breath) and the observed water concentration. For this we may turn to a previously developed simple linear model (19), with an extension to this case of dermal exposure. The one-compartment model treats the body as a single compartment. A very simple way to view the SC is to regard it as a membrane of infinitesimal thickness, whose only function is to impede the entry of chloroform into the blood for a certain lag time \( T \). (This parameter \( T \) has a value that is similar to the value of \( T_0 \) described above.) Under these conditions, the solution to the mass-balance differential equations for exposure to a constant concentration of chloroform in water is given by Wallace et al. (19):

\[
C_{\text{breath}} = 0 \ (0<t<T)
\]

\[
C_{\text{breath}} = f^+ C_{\text{water}} (1-e^{-(T-T_1)/\tau_{\text{uptake}}}) \ (t>T),
\]

(6)

\[
tau_{\text{uptake}}
\]

(7)

where \( C_{\text{breath}} \) is the chloroform concentration in the breath; \( C_{\text{water}} \) is the chloroform concentration in the water; \( f^+ \) is a constant relating the final equilibrium concentration in the breath to the concentration in the water; and \( \tau_{\text{uptake}} \) is the effective (uptake) residence time of the chemical in the body. \( C_{\text{breath}} \) is the mixed expired air, consisting both of alveolar air and the portion of the pure air supply that did not undergo alveolar exchange. (The alveolar concentration may be calculated from the whole breath concentration, using a nominal value for the anatomic dead space volume of the lungs.) \( \tau_{\text{uptake}} \) is expected to be affected somewhat by the fact that, in reality, the blood is not experiencing a constant exposure, but rather a rapidly increasing exposure for a short period immediately after the first measurable increase. Thus, \( \tau_{\text{uptake}} \) will probably be somewhat larger than the true residence time \( \tau \). The model has three parameters to be determined from the data: \( f^+ \), \( T \), and \( \tau_{\text{uptake}} \).

During the decay phase, the breath concentration declines exponentially:

\[
C_{\text{breath}} = Ae^{-t/\tau_{\text{decay}}},
\]

(8)

where \( t \) is the time measured from when exposure ends, \( A \) is the breath concentration when exposure ends, and \( \tau_{\text{decay}} \) is the effective residence time in the body during decay. \( \tau_{\text{decay}} \) is again expected to be affected somewhat (i.e., increased over the true residence time \( \tau \)) by the fact that the exposure experienced by the blood does not drop immediately to zero, but falls off at a certain rate determined by the characteristics of the SC. If exposure has lasted long enough to reach equilibrium, then the value of \( A \) should be given by \( f^+ C_{\text{water}} \).

The two-compartment model assumes a single metabolizing compartment and a second compartment, which is generally considered to include the organs or blood vessel-rich tissues (19). Again, the solution to continued exposure at a constant concentration is given by an initial period of zero breath concentration followed by a period of increasing concentration toward an asymptote:

\[
C_{\text{breath}} = 0 \ (0<t<T_1)
\]

\[
C_{\text{breath}} = f^+ C_{\text{water}} + A_1 e^{-(T-T_1)/\tau_{\text{uptake}}}
\]

\[
+ A_2 e^{-(t-T)/\tau_{\text{uptake}}} \ (t>T),
\]

(9)

(10)

where \( A_1, A_2 \) are lumped combinations of physiological parameters associated with the first and second body compartments; and \( \tau_{\text{uptake}}, \tau_{\text{delay}} \) are the effective (uptake) residence times of the chemical in the first and second body compartments. This model has six parameters: \( f^+, T, A_1, A_2, \tau_{\text{uptake}} \) and \( \tau_{\text{delay}} \). The decay phase for the two-compartment model is given by

\[
C_{\text{breath}} = A_1 e^{-t/\tau_{\text{delay}}} + A_2 e^{-t/\tau_{\text{uptake}}}.
\]

(11)

Again, if near-equilibrium has been reached, then \( A_1 + A_2 = f^+ C_{\text{water}} \).

All parameters were determined by fitting the background-corrected breath data using a nonlinear regression technique (Statgraphics Plus, Manugistics, Inc., Rockville, MD; or SigmaPlot Version 3.03, Jandel Scientific Software, San Rafael, CA). For the two-compartment model, only the decay phase was investigated in this way.

These simple models provide useful heuristic tools for interpreting the data. There may also be an advantage to applying physiologically based pharmacokinetic (PBPK) models to these data, and we plan to investigate this in future work.

Background breath concentrations were determined for each subject before and after exposure. If the final background concentrations were lower than the initial
background, which happened for subjects 1–4, a linear decrease of the background over that period was assumed. The calculated background concentrations were then subtracted from the observed breath concentrations to obtain a background-corrected value. These values were smoothed before they were finally used to calculate total exhaled amounts and to fit one- and two-compartment models.

Results

Multiple measurements of breath concentrations were made during and after dermal exposure to chloroform in domestic tap water at three temperatures. Figure 4 shows the breath chloroform concentration/time profiles obtained in this way for subjects 1 (male; Fig 4A) and 6 (female; Fig. 4B) at about 40°C. The plots show that, after dermal exposure commences, the concentration of breath chloroform rises quite rapidly and appears to level off after 10–20 min immersion in the water. Once the subject steps out of the bathwater and exposure ceases, the breath level begins to decrease and returns to a level close to the original background concentration. Similar breath uptake/elimination curves were obtained for all the remaining subjects at 40°C. The short ion storage times (500 msec) and high sampling frequency (6 sec per data point in the first stage and 12 sec in the second stage) used here allowed us to clearly define the critical early phases of the uptake and decay curves. Using conventional integrated sampling techniques, it would be difficult to assign a precise time when the decay phase begins because in uptake/decay exposure experiments such as these, some period of time typically elapses during the transition of the subjects from the exposed to the nonexposed state. In this study, however, the subjects were isolated from their immediate environment by the face mask and they breathed purified air throughout the entire experiment, so this problem did not arise.

The uncorrected plots in Figure 4 show measurements for breath chloroform at levels close to the detection limit of the system (low microgram per cubic meter range), and these weak ion beams give rise to considerable signal noise. There are several factors that play a role in reducing the measured ion signal. Unlike conventional GC/MS, in which sample preparation techniques typically include a sample pre-concentration step and measurement of the GC peak as a compressed plug, the breath analyzer used in the present study continuously consumes the compound during the analysis (35). At relatively constant, low sample concentrations, the analyte number density is small and this, in turn, limits the number of ions that will be formed in the glow discharge source (28). The ion injection efficiency into the ion trap from the glow discharge source has been estimated to be no more than 5% under optimum conditions (36). MS/MS conversion of parent ions to product ions further reduces the ion density in the trap by about a factor of 2, and more ion losses occur between the trap and the detector.

To minimize the deleterious effects of the scatter on the data from the dermal absorption of chloroform at different bathwater temperatures, the data were smoothed using a 60-sec block averaging time. Uptake experiments were conducted at three nominal temperatures, 30°C, 35°C, and 40°C, and the results obtained with one male and one female subject are presented in Figures 5A and B. The plots show that the breath levels hardly change from preexposure levels during exposure at the low (30°C) temperature. The levels are slightly higher at the medium (35°C) temperature, with the male subject (Fig. 5A) showing a somewhat larger increase than the female (Fig. 5B). At the high (40°C) temperature, the levels increase markedly and appear to level off at about the same maximum values reached in the experiments conducted at 40°C in the first phase of the study.

The amount of chloroform exhaled during the exposure and postexposure periods...

Figure 4. Continuous exhaled air concentration of chloroform as a function of time for two subjects during and following dermal exposure while bathing. (A) For subject 1 (male), exposure occurred for 24.4 min and postexposure monitoring ensued for 45.4 min; bathwater chloroform concentration was 91 µg/l and bathwater temperature was 41°C. (B) For subject 6 (female), exposure occurred for 30 min and postexposure monitoring ensued for 39 min; bathwater chloroform concentration was 92.5 µg/l and bathwater temperature was 39.6°C.
was calculated by integrating the area under the background-corrected curve. A respiration rate of 9 l/min for males and 7.5 l/min for females was assumed (37). Results, grouped by water temperature, are presented for all of the subjects in Table 2. The mean amount of chloroform exhaled varied from 0.2 ± 0.3 µg [mean ± standard deviation (SD)] at the low (30°C) temperature to 2.3 ± 1.4 µg at the moderate (35°C) temperature and 7.0 ± 2.0 µg at the high (40°C) temperature.

For the one-compartment model, estimates of $f^*$, $T_0$, $\tau_{\text{ uptake}}$, and $\tau_{\text{ decay}}$ are summarized in Table 3. All parameter estimates were highly significant ($p<0.01$) and $R^2$ values were in the range of 90%. For the two-compartment model, estimates of $A_1$, $A_2$, $\tau_{\text{ decay}}$, and $\tau_{\text{ depth}}$ are also provided in Table 3. Estimates of $A_1$ and $\tau_{\text{ decay}}$ were again significant ($p<0.05$) for all subjects, but estimates of $A_2$ and $\tau_{\text{ depth}}$ were significant for only 3 of the 10 subjects.

Because of the improved analytical techniques for the last six subjects, an attempt was made to arrive at a single normalized mean uptake and decay curve for all six (Fig. 6). The mean of the final five breath measurements during the exposure period was normalized to unity for each subject, and the initial breath measurement after exposure ended was also set to unity for each subject. One- and two-compartment fits to the mean values across all six subjects of their normalized data were then attempted. For the uptake curve, only a one-compartment fit, with a residence time $r_{\text{ uptake}}$ of 10.8 ± 1.0 min [mean ± standard error (SE)] was acceptable (adjusted $R^2 = 0.968$). The calculated value for the coefficient of the exponential function (Eq. 7) was 1.067, suggesting that the subjects had reached about 94% of their equilibrium value after 30 min exposure. For the decay curve, a two-compartment model appeared to fit the data better (adjusted $R^2 = 0.992$) than the one-compartment model (adjusted $R^2 = 0.966$); however, the values for $A_2$ and $\tau_{\text{ decay}}$ failed to reach significance. The one-compartment fit yielded a residence time $\tau_{\text{ decay}}$ of 7.7 ± 0.37 min (mean ± SE). For the two-compartment fit, $A_1 = 0.91 ± 0.06$ (SE) and $\tau_{\text{ decay}} = 0.1 ± 0.06$ (SE), suggesting that about 10% of the total body burden resided in the second compartment when exposure ended. In this case, $\tau_{\text{ decay}} = 5.28 ± 0.33$ min (SE) and $\tau_{\text{ depth}} = 221 ± 79$ min (SE).

The breath uptake curves at the high (40°C) temperature were also used to estimate the lag time $T_0$, which is measured from the time the subject steps into the water until the blood level of chloroform rises to a detectable level above background in the breath, i.e., to the time of the first measurable increase in the breath concentration. These values are summarized in Table 3. From these results, the average time to the first measurable increase is 4.7 ± 2.1 min (mean ± SD; range 2–8 min). A considerable difference in the value of $T_0$ was observed for the first group of four subjects (6.78 ± 1.01 min) compared to the second group of six subjects (2.75 ± 0.57 min). The latter value may be more reliable because of the improved analytical techniques and higher sensitivities available in the second phase of the study.

**Discussion**

The most striking result of these studies is the very strong effect of bathwater temperature on dermal absorption of chloroform. The mean exhaled amount of chloroform increased from 0.2 µg at the lowest bathwater temperature to 7 µg at the highest temperature, a factor of about 30. At the lowest temperatures (28–32°C), virtually no chloroform was absorbed by four of six subjects, despite a full 25–30 min exposure, and only a two-µg value was exhaled by the other two. At slightly higher temperatures (34–36°C), the amount exhaled increased by an order of magnitude, to 2.3 ± 1.4 µg (mean ± SD). At the highest temperatures used (38–41°C), the amount exhaled increased by another factor of 3, to 7.0 ± 2.0 µg. Similarly, the average maximum observed breath concentrations increased from 3 to 19 to 45 µg/m³ for the three temperature regimes.

We believe the likely explanation for this effect is the heat-conserving or heat-dissipating mechanisms of the body. At low
bathwater temperatures, the capillaries closest to the skin surface experience greatly lessened blood flow. This effectively forces the chloroform to diffuse across a greater distance to reach the blood. Also, the lower temperature of the SC may cause a lessening of the speed of diffusion, a process highly dependent on temperature (38). At high bath temperatures, increased blood flow to the skin results in a shorter effective diffusion length and an increased speed of diffusion. If blood-flow changes to the skin are responsible for these large changes in dose, we should expect to find temperature-related blood-flow changes of at least this order of magnitude. In fact, measurements of blood flow in the extremities have shown even larger factors of 80–600 for blood flow through the fingers on changing from a cold to a hot environment (39).

These findings help to explain certain results obtained in previous studies. For example, Jo et al. (6) found that for six subjects taking 13 showers with and without wet suits, the amount of chloroform exhaled during normal showers was about twice the amount exhaled during the showers with wet suits (inhalation only), suggesting that the dermal route contributed about 50% of the total dose. However, a study of swimmers by Lévesque et al. (13) found that only about 25% of the exposure was due to dermal absorption. Finally, in a recent study of swimmers undergoing rigorous training, Lindstrom et al. (14) concluded that the dermal pathway was responsible for about 75% of the total exposure to chloroform. These different findings all suggest that the temperature/blood flow relationship is paramount in determining dermal absorption. In the first case, all showers were at high temperatures, leading to a large dermal effect. In the

### Table 2. Exhaled chloroform dose for subjects in bathwater at three temperatures

| Experiments | Subject | Water temperature (°C) | Chloroform concentration in water (μg/l) | Time in bath (min) | Max obs breath conc (μg/m³) | Exhaled dose (μg) |
|-------------|---------|------------------------|------------------------------------------|--------------------|----------------------------|------------------|
| Low temperature | 7 | 28.2 | 89.5 | 30 | 3 | 0.0 |
| 8 | 28.8 | 88 | 29.2 | 0 | 0.0 |
| 9 | 29.5 | 101 | 30 | 1 | 0.0 |
| 4 | 30 | 81 | 15 | 8 | 0.6 |
| 6 | 32.1 | 86.5 | 30 | 2 | 0.0 |
| 5 | 32.4 | 54.5 | 25.4 | 5 | 0.5 |
| Mean ± SD | 30.2 ± 1.6 | 83.4 ± 14.3 | 26.6 ± 5.4 | 3.2 ± 2.7 | 0.2 ± 0.3 |
| Medium temperature | 8 | 33.8 | 93.5 | 30 | 6 | 0.6 |
| 7 | 34.5 | 94.5 | 29.8 | 30 | 3.9 |
| 9 | 34.8 | 91 | 30 | 13 | 1.3 |
| 6 | 35.0 | 90.5 | 30 | 10 | 1.1 |
| 10 | 35.7 | 92.5 | 29.8 | 30 | 3.6 |
| 5 | 36.0 | 78 | 32.2 | 25 | 3.6 |
| Mean ± SD | 35.0 ± 0.7 | 90.0 ± 5.5 | 30.3 ± 0.9 | 19.0 ± 9.7 | 2.3 ± 1.4 |
| High temperature | 8 | 38.3 | 98 | 29.8 | 30 | 4.2 |
| 7 | 38.3 | 98 | 25.2 | 60 | 8.7 |
| 9 | 38.8 | 98 | 25.2 | 45 | 6.9 |
| 10 | 39.0 | 93 | 29.6 | 70 | 9.6 |
| 6 | 39.6 | 92.5 | 30 | 70 | 9.2 |
| 5 | 39.7 | 93.5 | 21 | 45 | 7.9 |
| 2 | 40 | 91 | 26.9 | 20 | 4.2 |
| 3 | 40 | 91 | 26.9 | 32 | 7.4 |
| 4 | 40 | 81 | 27.5 | 41 | 6.9 |
| 1 | 41 | 91 | 24.4 | 43 | 8.4 |
| 4 | 41 | 81 | 27.3 | 38 | 4.1 |
| Mean ± SD | 39.6 ± 0.9 | 85.8 ± 15.6 | 27.4 ± 2.6 | 44.9 ± 15.3 | 7.0 ± 2.0 |

SD, standard deviation.

*Maximum observed chloroform concentration measured in mixed expired air.

*Calculated using 9/min respiration rate for males and 7.5/min for females.

### Table 3. Theoretical calculations of model parameters

| Model | Parameters | Subject | Mean ± SD |
|-------|------------|---------|-----------|
|       |            | 1       | 2         | 3         | 4         | 5         | 6         | 7         | 8         | 9         | 10        |
|       |            |         |           |           |           |           |           |           |           |           |           |
| Chloroform in water (μg/l) | 91 | 91 | 91 | 81 | 39.5 | 93 | 88 | 98 | 98 | 93 | 86.4 ± 16.3 |
| Exposure time (min) | 24.4 | 26.9 | 26.9 | 27.5 | 21 | 28 | 29.2 | 29.8 | 29.2 | 29.6 | 27.3 ± 2.6 |
| Chloroform concentration (ng/ml) | 0.48 | 0.22 | 0.35 | 0.5 | 1.06 | 0.70 | 0.52 | 0.30 | 0.65 | 0.58 | 0.54 ± 0.24 |
| Chloroform concentration in water (μg/l) | 7.6 | 6.6 | 5.2 | 7.7 | 2.4 | 1.8 | 3.6 | 2.8 | 3.2 | 2.7 | 4.4 ± 2.1 |
| Max breath conc (μg/m³) | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 |
| Chloroform concentration in water (μg/l) | 5.7 | 14 | 12 | 15 | 9.8 | 27 | 24 | 20 | 20 | 21 | 17 ± 7 |
| Chloroform concentration in water (μg/l) | 0.48 | 0.22 | 0.35 | 0.5 | 1.06 | 0.70 | 0.52 | 0.30 | 0.65 | 0.58 | 0.54 ± 0.24 |
| Chloroform concentration in water (μg/l) | 7.6 | 6.6 | 5.2 | 7.7 | 2.4 | 1.8 | 3.6 | 2.8 | 3.2 | 2.7 | 4.4 ± 2.1 |

**Abbreviations:** C_wamr, chloroform concentration in water; \( f' \), constant relating equilibrium concentration in breath to concentration in water; \( T_w \), time of immersion in water to first measurable increase in breath concentration; \( T_r \), residence time in 8th body compartment; \( A_t \), physiological parameter associated with 8th body compartment.

*Time to first increase, measured from initial immersion in water until breath concentration shows first measurable increase (3 ± standard deviation above background).

*Time required for breath concentration to reach 87% (2σ) of maximum value.

*All \( A_t \) and \( T_{decay} \) values are significant.

*Only subjects 1, 4, and 6 had significant \( A_t \) and \( T_{decay} \) values.

*\( T_{decay} \) value for subject 10 is not included in statistics.

Environmental Health Perspectives • Volume 106, Number 6, June 1998
not exercise in swimming pools would be expected to produce even less dermal absorption. We tested one person in a swimming pool at a temperature of 29°C and a chloroform concentration of 70 μg/l and found no increase in breath chloroform after 25 min exposure at low activity. Although the water temperature in Lindstrom et al.’s (14) experiment was also lower than that of a normal shower, the vigorous aerobic training had the same effect as the warmer water temperature by increasing the internal (core) temperature, which resulted in greatly increased blood flow and therefore in increased dermal absorption.

We can calculate the portion of the dose that resides in the reservoir of the SC (after equilibrium is attained) by multiplying the average concentration \( \bar{C}_{water} \) from Equation 5 by the volume of the SC. If 90% of the skin surface (about 16,000 cm²) is in contact with the water and if the average thickness \( L \) of the SC is 10 μm, then the volume of the submerged part of the SC is 16 cm³ and the mass of chloroform in the SC will be \( 16(\bar{C}_{water} \times 0.5 \times 0.001) \). For a skin–water partition coefficient of 10 and a nominal chloroform concentration of 100 μg/l, the amount of chloroform in the SC would be 8 μg. If the average thickness of the SC is 25 μm and if \( P_{sc} = 24 \) [upper limit noted by McKone (40)], then the amount in the SC would be 48 μg.

From Equations 1–5, a characteristic time \( \tau_{sc} \) (shown in Fig. 3) may be obtained, with the model of McKone (40) and the 29 min estimated by an EPA model discussed by McKone (40).

The third portion of the S-shaped curve, the time to approach the asymptote, may be estimated from the single-compartment model uptake residence times \( \tau_{upake} \) listed in Table 3. If we use the uptake model to define the time to reach the asymptote, we can express this time in terms of a simple multiple of \( \tau_{upake} \). For example, if the breath concentration is equal to 87% of the maximum value at a time corresponding to \( 2\tau_{upake} \). Applying this condition to the data in Table 3, the times to reach the asymptote, also summarized in Table 3, are between 10 and 27 min for all subjects except subject 1 (6 min). It should be noted that these times to reach the breath asymptote are not the same as the time required to reach the flux asymptote, as shown in Figure 3.

The one-compartment model was fitted to the uptake and decay data for all of the subjects, and values obtained for \( \tau_{upake} \) and \( \tau_{decay} \) are presented in Table 3. The mean residence times for the uptake phase are quite similar to the times for the decay phase, namely, \( \tau_{upake} = 8.2 \pm 3.1 \) min (mean ± SD) and \( \tau_{decay} = 7.7 \pm 1.0 \) min. Using the two-compartment model for the decay phase, the mean residence time for the first compartment \( \tau_{1,decay} \) is a little smaller (5.3 ± 1.7 min) than the mean obtained for the one-compartment model. However, the estimates of \( \tau_{2,decay} \) were significant for only 3 of the 10 subjects. These results suggest that the decay curves for the two compartments are being obscured by the continuous diffusion of chloroform through the SC for a time after exposure ends. That is, there is not a clear bi-exponential decline as observed in previous inhalation-only studies (18,19,24,42). This continued influx would have the effect of increasing the observed residence time in the first compartment. So, for example, our observed times of 6–9 min for the decay phase are somewhat larger than the times of 1–7 min obtained for chloroform in other studies (16,18,42,43). These very short residence times underscore the importance of the first few minutes immediately following the onset or cessation of exposure and demonstrate why measurements need to be made with high frequency in order to determine the true shape of the uptake and decay curves.

We can relate these theoretical considerations to our observed parameter (exhaled breath concentration) by noting that the dose during the exposure must be at least equal to the total amount of chloroform exhaled over the measurement period. In
fact, since some chloroform is metabolized, the amount exhaled is only a fraction of the total dose. However, the magnitude of this fraction is not clear. Observations made with other nonvolatile VOCs suggest a range between 0.1 and 0.3 (19,42).

Conclusions
This study has shown that water temperature exerts a very strong effect on dermal absorption of chloroform while bathing. The study has also successfully demonstrated the potential of the real-time breath analyzer for monitoring rapid changes in concentration that would otherwise be difficult to detect using more conventional time-integrated sampling techniques with standard GC/MS analysis. The technique has allowed us to determine the time it takes for chloroform to first appear at a measurable level in the exhaled breath after diffusing through the skin during bathing. It has also provided an estimate of the resulting breath concentration at near-equilibrium as a function of the chloroform concentration and water temperature.

References and Notes
1. IARC. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans. Vol. 52: Chlorinated Drinking-Water; Chlorinated By-Products; Some Other Halogenated Compounds: Coal and Cobalt Compounds. Lyon:International Agency for Research on Cancer, 1991.
2. Cantor KP, Hoover R, Hartge P, Mason TJ, Silverman DT, Altman R, Austin DF, Child MA, Key CR, Marratt LD, et al. Bladder cancer, drinking water source, and tap water consumption: a case–control study. J Natl Cancer Inst 79:1269–1279 (1987).
3. Vena JE, Graham S, Freudenberg J, Marshall J, Zlatezky M, Swanson M, Sufin G. Drinking water, fluid intake, and bladder cancer in western New York. Arch Environ Health 49:191–198 (1993).
4. Morris RD, Audet AM, Angelillo IF, Chalmers TC, Mosteller F. Chlorination, chlorination by-products, and cancer: a meta-analysis. Am J Public Health 82:955–963 (1992).
5. Bove F, Fulcomer MC, Savrin JE. Public drinking water contamination and birth outcomes. Am J Epidemiol 141:850–862 (1995).
6. Jo WK, Weisel CP, Lioy PJ. Routes of chloroform exposure and body burden from showering with contaminated tap water. Risk Anal 10:575–580 (1990).
7. Andelman JB. Human exposures to volatile halo- genated organic chemicals in indoor and outdoor air. Environ Health Perspect 82:313–318 (1985).
8. Weisel CP, Jo WK, Ingestion, inhalation, and dermal exposures to chloroform and trichloroethene from tap water. Environ Health Perspect 104:48–51 (1996).
9. Wester RC, Malbach H. Percutaneous absorption of chemicals from water simulating swimming and bathing and from vapor exposure. Environ Sci Pollut Control Ser 9:149–165 (1994).
10. Wallace LA, Sheldon L, Hartwell TD, Zelon H, Perritt R, Sparacio C, Pellizzari ED. Exposures of swimming pool lifeguards to chloroform and other volatile organic chemicals: measurements in air, water, breath, blood, and urine. Paper presented at the 2nd International Conference on Total Exposure Assessment Methodology (TEAM), 18–22 November 1991, Atlanta, GA.
11. Agazzotti G, Fantuzzi G, Righi E, Tartoni P, Cassignadri T, Predieri G. Chloroform in air of individuals attending indoor swimming pools. Arch Environ Health 48:250–254 (1993).
12. Weisel CP, Shepard TA. Chloroform exposure and the body burden associated with swimming in chlorinated swimming pools. In: Water Contamination and Health. New York:Marcel Dekker, 1994:135–147.
13. Lévesque B, Ayotte P, LeBlanc A, Dewailly É, Prud'Homme D, Levaière R, Beaton A, Levallée P. Evaluation of dermal and respiratory chloroform exposures in humans. Environ Health Perspect 102:1082–1087 (1995).
14. Lindstrom AB, Pei JD, Berkoff DC. Alveolar breath sampling and analysis to assess trichloroethane exposures during competitive swimming training. Environ Health Perspect 105:436–442 (1997).
15. Wallace LA, Buckley T, Pellizzari E, Gordon S. Breath measurements at a volatile organic compound bio-markers. Environ Health Perspect 104 (suppl 5):861–869 (1996).
16. Weisel CP, Jo WK, Lioy PJ. Utilization of breath analysis for exposure and dose estimation of chloroform. J Expo Anal Environ Epidemiol Suppl 1:55–69 (1992).
17. 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).
18. Gordon SM, Jo WK, Pei JD. Continuous real-time breath analysis for the measurement of half lives of expired volatile organic compounds. J Expo Anal Environ Epidemiol Suppl 1:94–104 (1992).
19. Wallace LA, Lioy PJ. An algorithmic model relating breath concentrations to environmental exposures: application to a chamber study of four volunteers exposed to volatile organic chemicals. J Expo Anal Epidemiol Epidemiol 37:102–103 (1993).
20. Kelly TJ, Pei JD, Veldman GM. Continuous analysis of human breath using atmospheric pressure ionization MS/MS with a novel inlet design. In: Proceedings of the 1989 EPA/A&WMA International Symposium on Measurement of Toxic and Related Air Pollutants, VIP-13, Pittsburgh, PA:Air & Waste Management Association, 1989:478–483.
21. Kelley PE. Mass Spectrometry Method Using Nuch Filter. U.S. Patent 5,134,286, 28 July 1992.
22. Kelley P, Hoekman D, Bradshaw S. A new method of operation for the quadrupole ion trap. In: Proceedings of the 41st ASMS Conference on Mass Spectrometry and Allied Topics, 31 May–4 June 1993, San Francisco, CA. Santa Fe, NM/ASMS, 1993:453–458.
23. Kenny DV, Callahan PJ, Gordon SM, Stillier SW. Simultaneous isolation of two different m/z ions in an ion trap mass spectrometer and their tandem mass spectra using filtered noise fields. Rapid Commun Mass Spectrom 7:1086–1089 (1993).
24. Gordon SM, Wallace LA, Pellizzari ED, O'Neill HJ. Human breath measurements of chloroform in air chamber to determine half lives for volatile organic compounds. Atmos Environ 22:2165–2170 (1988).
25. U.S. EPA. The Determination of Halogenated Chemicals in Water by the Purge and Trap Method. Method 502.1, EPA/600/R-81-059. Cincinnati, OH: U.S. Environmental Protection Agency, 1981.
26. McLuckey SA, Glish GL, Asano KG, Grant BC. Atmospheric sampling glow discharge ionization source for the determination of trace organic compounds in ambient air. Anal Chem 60:2229–2227 (1988).
27. McLuckey SA, Glish GL, Asano KG. The coupling of an atmospheric sampling ion source with an ion trap mass spectrometer. Anal Chem Acta 225:35–35 (1989).
28. Asano KG, McLuckey SA, Glish GL. Comparison of atmospheric sampling glow discharge ionization with electron ionization. Spectroscopy 8:191–209 (1990).
29. Burton SM, Glish PL, McLuckey SA. Direct trace analysis of volatile organic compounds in air using ion trap mass spectrometers with filtered noise fields. In: Proceedings of the 1995 EPA/A&WMA International Symposium on Field Screening Methods for Hazardous Wastes and Toxic Chemicals, Vol. 1. VIP-47. Pittsburgh, PA:Air & Waste Management Association, 1995:670–673.
30. Wallace LA, The TEAM Study: Summary and Analyses. Volume I. EPA/600/R-90/002a. Washington, DC:U.S. Environmental Protection Agency, 1987.
31. Duckworth DC, Smith DH, McLuckey SA. Improved signal-to-noise ratio in glow discharge ion trap mass spectrometry via pulsed discharge operation. J Anal At Spectrom 12:43–48 (1997).
32. Winberry WT Jr, Murphy NT, Riggins RM. Methods for Determination of Toxic Organic Compounds in Air: EPA Methods. Park Ridge, NJ:Noyes Data Corporation, 1990.
33. Pirrò F, Kalin YN, Stinchcomb AL, Keating G, Bunge A, Guy RA. Characterization of the permeability barrier of human stratum corneum. Proc Natl Acad Sci USA 94:1562–1567 (1997).
34. Carslaw HS, Jaeger JC. Conduction of Heat in Solids. Oxford, UK:Clarendon Press, 1955.
35. LeBlanc JD, McLuckey SA. Mass Spectrometry/Mass Spectrometry: Techniques and Applications of Tandem Mass Spectrometry. New York:VCH Publishers, 1988.
36. McLuckey SA, Goeringer DE, Asano KG, Vaidyanathan G, Stephenson JL Jr. High explosives vapor detection by glow discharge ion trap mass spectrometry. Rapid Commun Mass Spectrom 10:287–298 (1996).
37. U.S. EPA. Exposure Factors Handbook, Vol. I: Review Draft. EPA/600/R-95/032a. Washington, DC:U.S. Environmental Protection Agency, 1996.
38. Glastone SL, Laidler KJ, Eyring H. Volatility and diffus- ion. In: The Theory of Rate Processes. New York:McGraw-Hill, 1941:477–551.
39. Mountcastle VB. Medical Physiology Volume Two. St. Louis, MO:C.V. Mosby, 1980.
40. McKone TE. Linking a FPBK model for chloroform with measured breath concentrations in showerers: implications for dermal exposure models. J Expo Anal Epidemiol 3:339–365 (1993).
41. Crank J. Diffusion in a plane sheet. In: The Mathematics of Diffusion. Oxford, UK:Clarendon Press, 1956:42–41.
42. 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 Epidemiol 7:141–163 (1997).
43. Pei JD, Lindstrom AB. Exhaled human breath measure- ment method for assessing exposure to halo- genated volatile organic compounds. Clin Chem 43:723–730 (1997).