Alveolar sampling and fast kinetics of tetrachloroethene in man. I Alveolar sampling

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ABSTRACT Human subjects were exposed to tetrachloroethene (perchloroethylene, PER). The duration of exposure ranged from one to 60 minutes and the concentration of PER in inhaled air ranged from 0.02 to 0.40 mmol/m³. Alveolar air was sampled after several residence times (t^*) in the lung. Both during and after exposure, the concentration of PER in alveolar air (C_{Alv}) as a function of the residence time was studied to estimate the concentration in the pulmonary artery (C_{Ven} : mixed venous blood) and in the pulmonary vein (C_{Art} : arterial blood). During exposure C_{Alv} decreased as function of t^* . At $t^* = 10$ s C_{Alv} was 70–75% of the value presented at $t^* = 5$ s; this decrease approximates an exponential curve. C_{Alv} seemed to stabilise at $t^* = 10$ –12 s, whereas it decreased more rapidly at $t^* > 12$ s; this decrease continued up to at least $t^* = 55$ s when C_{Alv} was about 40% of the value it represented at $t^* = 5$ s. In the postexposure period C_{Alv} increased as function of t^* from 5 to 10 s. Both during and after exposure, no difference was observed between C_{Alv} at $t^* = 10$ s and C_{Alv} in the exhaled part of the expiratory reserve volume. A simple gas exchange model showed that the decrease or increase of C_{Alv} at $t^* < 10$ s could be explained by either absorption or excretion by mixed venous blood. C_{Alv} at $t^* = 10$ –12 s provided a valid estimate of C_{Ven} . To estimate C_{Art} , the fluctuating character due to the discontinuous breathing with a breathing frequency had to be taken into account. It is shown that C_{Alv} during normal breathing ($t^* = 5$ s) provides a reasonable estimate of the time weighted concentration in arterial blood.

In biological monitoring for several solvents a relation has been studied between external level of exposure and a biological concentration (in blood, urine, or breath, for example) for a group of workers.^{1,2} This relation is usually poor because of uncertainties in, for example, inter/intraindividual differences in the kinetic behaviour of the agent or its metabolites.

Human exposure experiments are suitable for studying the individual kinetics by measuring the course of concentration of agents/metabolites in breath or blood. To study the individual kinetic behaviour one has to account for kinetic phenomena ranging from fast to slow. The fast kinetics concern the distribution among rapidly perfused organs/tissues such as the brain or the kidneys; they are important during peak concentrations and in the early postexposure period. Studying fast kinetic phenomena in experiments can be helpful in more detailed compartment modelling in which circulation times may play an important part.³ The slow kinetics

concern the absorption or desorption in the slowly perfused organs/tissues—for example, the fat; they are most important in a late postexposure period.

To study the fast kinetic behaviour, the blood in the pulmonary artery seems to be one of the most important biological media. Blood in the pulmonary artery is mixed venous blood composed of all types of local venous blood and therefore of all contributions from perfused organs/tissues with different concentrations.

The purpose of our investigation was to study optimal alveolar air sampling conditions in order to estimate the concentration in mixed venous blood during and after exposure to solvents, with the aid of accurate sampling and analysis of alveolar air.

Subjects were exposed to tetrachloroethene vapour (PER). This solvent was chosen because it has a blood-air partition coefficient of the same order of magnitude as many other solvents; sampling conditions therefore do not deviate largely from those applying to other solvents.⁴ In addition, a highly accurate analysis of PER may be obtained down to low concentrations using gas chromatography with

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an electron capture detector; this allows the exposure of subjects to concentrations of PER far below the TLV (335 mg/m^3). From the kinetic point of view, PER is relatively easy to describe as it is scarcely metabolised and is almost totally excreted by exhalation.^{5,6}

Methods

PROCEDURE

In a period of six months six volunteers (3 men, 3 women) were exposed at rest several times to different concentrations of tetrachloroethene (Perchloroethylene, PER).

Concentrations in the inhaled air ranged from 0.02 to 0.04 mmol/m^3 (0.5–9 ppm) and exposure lasted from one to 60 minutes. The respiratory frequency (f) was about 12/min and the minute volume of ventilation (MV) about 5–8 l. Subjects were in the sitting position and they breathed quietly through a valve out of a Tedlar bag; only when a sample was taken did the subjects hold their breath for some seconds before exhalation. After this exhalation, a sample of the last part of alveolar air was collected. At the end of exposure the bag was shut off and the subjects immediately inhaled fresh air.

Different alveolar air sampling methods were used during and after exposure: *method 1 (normal)*: normal inhalation followed by normal exhalation;

method 2 (breath holding): normal inhalation followed by breath holding for 1–50 s and normal exhalation; *method 3 (reserve volume)*: after a normal inhalation and exhalation, the subjects exhaled part of the expiratory reserve volume which had about the same volume as that of normal exhalation (about 0.4 l).

The postexposure period lasted for a further three days. To maintain the same experimental conditions in the early postexposure period as during exposure, the subjects breathed fresh air through the valve for the first 10 minutes after exposure.

APPARATUS

A Tedlar polyvinylfluoride bag (capacity 400 l) was connected to a glass tube system (id 21 mm), which contained a valve, a flowmeter and a gas volume meter (fig 1). The parts of the glass tube system were coupled by insertion into Teflon connectors which contained Viton O-rings. The Tedlar bag contained air of 20°C , with a concentration of PER and a relative humidity of 60%. The concentrations of PER were produced by a standard generator (Analytical Instrument Development Inc, model 350).

During exposure, samples of inhaled air were taken with a vacuum sampling tube plugged into the tube system at location A. The concentration of PER in the inhaled air was constant to within 2%. The valve is a one way Ruben valve (R & S Denmark) with a

Fig 1 Diagram of apparatus. Subjects breath through mouth piece of valve while wearing noseclip. Inhaled air is obtained from Tedlar bag and exhaled air leaves system through gas volume meter. Vacuum sampling glass tube may be plugged into the system at locations A and B to sample inhaled or exhaled air respectively.

dead volume of 10 ml and a pressure resistance of 135 Pa (1 mm Hg) to normal expiration and inspiration. The valve is made from aluminium and polycarbonate (macrolon) and the moving part inside the valve is made from nylon 11 (Rilsan); absorption and desorption were not observed. The valve and the tube system were heated to 37°C to avoid condensation of exhaled air.

After exhalation, samples of the last part of alveolar air could be collected at location B with the help of vacuum sampling tubes (45 ml); the vacuum tube was opened for two seconds. These vacuum tubes were kept at 37°C. The temperature of the exhaled alveolar air at location B was about 34.5°C. When the exhaled air had passed location B, it flowed through a flow meter and a gas volume meter (Wilson G4). The flow meter (Monaghan 403, pulmonary function analyser) registered the expired air flow continuously, thus allowing a check of the breathing frequency, time of breath holding, and irregularity of breathing. In combination with the gas volume meter, it was possible to determine the total volume and to recognise possible hyper/hypoventilation. Before the air entered the gas volume meter, it was cooled to room temperature to avoid corrosion. The pressure resistance of the whole system to normal expiration was about 270 Pa (2 mm Hg).

At the end of exposure, the cock in the glass tube system was closed and the cap unscrewed: the subject immediately inhaled fresh air.

Before sampling, the pressure in the vacuum sampling tube was less than 270 Pa (2 mm Hg). The tube was sealed by a PTFE-Teflon stopcock (Quickfit, Rotaflor). The dead space of the bore was 0.3% of the sampling volume. A screwcap sealed one end of the tube. This cap had a predrilled hole for gas sampling, a silicon rubber liner (septum), and a smooth Teflon

liner that sealed the glass tube to prevent absorption of PER into the silicon rubber.

ANALYSIS

The PER concentrations in alveolar air samples were stable for at least five days at room temperature. During analysis, the sampling tubes were kept at 37°C to avoid condensation and changes in air pressure. The cooling and subsequent heating did not affect the results. By means of a gas tight precision syringe (Hamilton), a volume of 25 μ l was injected into a gas chromatograph that was fitted as follows: glass column: length 170 cm, 3 mm id, 115°C, N₂ flow of 30 ml/min; stationary phase: 4% OV17 on Gaschrom Q 80/100 mesh; injector: 150°C; detector: ⁶³Ni ECD, 320°C; instrument: Packard 428.

Calibration curves were prepared by the standard generator, this instrument used capillary diffusion tubes of constant temperature. By means of dilution with an air stream, all desired concentrations over a certain range could be obtained to within 1%. The air stream was saturated with water at 37°C.

We analysed the samples with a standard deviation of 1–2%; the value of this SD was determined by sampling and analysing the water saturated air stream with a constant concentration of PER coming from the standard generator. The detection limit was about 0.006 μ mol/m³ (1 μ g/m³). Correction to BTPS conditions did not affect the results.

Results

COURSE OF ALVEOLAR CONCENTRATION

Samples were taken after different periods of breath holding. Because the last part of the exhaled air was collected, a distinction has to be made between the time of breath holding and the residence time in the lung. The time of breath holding refers to the time interval between the end of inhalation and the start of exhalation. The residence time (t^*) is defined as the time interval between the beginning of inhalation and the end of the next exhalation—that is, the time in which gas exchange with mixed venous blood can take place (fig 2). In this paper all samples taken after a certain breath holding time are expressed with their corresponding residence times. At a breathing frequency of 12/min, a zero breath holding time corresponds to a residence time of $t^* \approx 5$ s, whereas a sample of the reserve volume corresponds to $t^* \approx 7.0$ s.

Figure 3 shows, for each subject, an example of the sample points with the three methods of sampling, only during the first 55–60 minutes after the onset of an exposure. Figure 4 shows an overall view of the results of the exhaled alveolar concentration (C_{AIV}) relative to the inhaled concentration during exposure.

Fig 2 Schematic view of flow during inhalation and exhalation. During normal breathing—that is, breath holding time $t^{**} = 0$ —at a respiratory frequency of $f = 12/\text{min}$, residence time $t^* \approx 5$ s; at end of inhalation: $t^* \approx 2.5$ s. Underbroken flow represents an extra exhalation from expiratory reserve volume; this yields $t^* \approx 7.0$ s. Breathing with breath holding ($t^{**} \neq 0$) yields a longer residence time—for instance, 5 s breath holding time yields $t^* = 10$ s.

Fig 3 Examples of time courses of relative PER concentration in alveolar air during 55–60 minutes after onset of exposure for six subjects. Symbols: $\circ$ = normal (zero breath holding); $t^* = 4-6$ s. $\square$ = 2 s breath holding; $t^* = 6.5-8.5$ s. $\bullet$ = reserve volume; $t^* = 5-7$ s; $\times$ = 5 s breath holding; $t^* = 8-12$ s. ∇ = 7 s breath holding; $t^* = 11.5-13$ s. $\blacktriangle$ = 15 s breath holding; $t^* = 18-21$ s; t_1 = duration of exposure.

Fig 4 Overall view of alveolar concentration curves relative to inhaled concentration (C_I) of PER at different residence times (t^*). In postexposure period lines are drawn schematically; at $t > 240$ min no difference was observed between sampling with $t^* = 10$ s and $t^* > 10$ s.

(C_I) at different residence times. During exposure, the curves were obtained by curve fitting the sample points with the same t^* using a summation of two exponential terms which corresponds to a two compartment model.⁷ Although there was an intra- and interindividual variability in the level of C_{Alv} , the ratios between the C_{Alv} values with different residence times reproduced well in all experiments. Immediately after the end of exposure, the alveolar concentration decreased rapidly.

SAMPLING ERRORS

In addition to the error in the analysis (1–2%), an additional error might be introduced by the method of sampling. In practice t^* could not be fixed—for example, sampling after zero breath holding time was carried out with a measured $t^* = 4.0 - 6.0$ s. In addition, it was observed that during exposure, an increase or decrease in the minute volume of ventilation (MV) caused respective increases or decreases within 20 s in the course of C_{Alv} . Under these conditions, during a single exposure the weighted residual errors of the measured concentrations around the individual two exponential fitted curve at the 5 s, 10 s, and 20 s residence time and the reserve volume were about 5%.†

After exposure the residual error in the measured concentrations around an exponential fitted line of 5 s, 10 s, 20 s residence times, and of the reserve volume, was about 2.5%. During the postexposure period triplo samples were within 3%.

EFFECT OF BREATH HOLDING ON ALVEOLAR CONCENTRATION DURING EXPOSURE

Figure 4 illustrates that the alveolar concentration strongly depends on the residence time (t^*). Sampling was carried out in detail with subjects a and b with an exposure duration of $t > 14$ minutes. In each experiment samples were taken at several times during exposure with several residence times between 4.8 and 26 s; for each value of t^* , sampling took place three to eight times. By means of curvilinear interpolation, the alveolar concentration for a certain t^* was estimated at an exposure time point ($t = 14$ min). Figure 5 shows the course of $C_{Alv}(t^*)/C_I$ in eight experiments on subject a as a function of the residence time (t^*) at the exposure time point of 14 minutes. The value of C_{Alv}/C_I at $t = 14$ min is not the same in the different experiments because of intraindividual variation.

Normal breathing ($t^* \approx 5$ s) yields the highest mea-

†The weighted residual error equals

$$\sqrt{\frac{1}{n-p} \sum_{i=1}^n \left(\frac{y_i - \hat{y}_i}{\hat{y}_i} \right)^2} \times 100\%$$

where y_i = measured value of a sample point ($i = 1, \dots, n$); $\hat{y}_i$ = calculated value by means of fitted curve with p parameters (in this case $p = 4$).

Fig 5 Examples of decrease of alveolar concentration (C_{Alv}) of PER relative to inhalation concentration (C_I) at exposure time $t = 14$ min for several experiments ($n = 8$) carried out with subject a. Each experiment is presented by a symbol.

sured alveolar concentration, but the alveolar concentration decreases rapidly with increasing t^* . If the t^* is increased from 5 to 6 s, this results in a decrease of the alveolar concentration of about 10%.

The alveolar decrease in the range of $t^* = 5-10$ s approximates an exponential curve up to about $t^* = 10$ s. At $t^* = 10$ s, the decrease of alveolar concentration is 20–25% compared to the value at $t^* = 5$ s. At $t^* > 10$ s the decrease of C_{Alv} continues. The concentrations of the reserve volume ($t^* = 7.0$ s) were found to be similar to C_{Alv} ($t^* = 10$ s) instead of C_{Alv} ($t^* = 7.0$ s). An example is shown for subject d in fig 3.

The same decrease of C_{Alv} was found when the subject exhaled one long breath in sequential parts (fig 6).

It is assumed that the fast decrease of the concentration of PER in alveolar air for $t^* < 10$ s is caused by the PER absorption by mixed venous blood and that the decrease at $t^* > 10$ s up to $t^* = 55$ s may be explained by fast distribution among fast perfused organs/tissues.⁸

EFFECT OF BREATH HOLDING ON ALVEOLAR CONCENTRATION AFTER EXPOSURE

To study conditions in which alveolar air and mixed venous blood equilibrate optimally, one has to consider the early postexposure period (< 30 min after exposure) and the later postexposure period (> 30 min after exposure) separately.

Fig 6 Examples of course of alveolar concentration as function of residence time t^* during one long exhalation during exposure with subject b. Symbols: Δ at $t = 14$ min; $\circ$ at $t = 26$ min during exposure 1; $\blacktriangle$ at $t = 14$ min; $\bullet$ at $t = 26$ min during exposure 2.

In the early postexposure period the ($t^* = 10$ s) values are the same as the reserve volume values but are 5% higher than the ($t^* = 5$ s) values. In the later postexposure period the change in mixed venous concentration is much slower, which allows for a study of the equilibration between C_{ven} and C_{Alv} . In the later postexposure period the ($t^* = 10$ s) and ($t^* = 5$ s) values decrease in a constant ratio: the ($t^* = 10$ s) values are 10% higher than ($t^* = 5$ s) values. In the later postexposure period no significant differences between reserve volume values ($t^* = 10$ s) values and ($t^* \geq 20$ s) values were observed (fig 4, C_{Alv} ($t^* = 10$ s) $\approx C_{Alv}$ ($t^* \geq 10$ s)).

Discussion

ALVEOLAR MIXED VENOUS EQUILIBRATION

In this study the concentration of PER in mixed venous blood (C_{ven}) was estimated by means of the alveolar concentration (C_{Alv}). In the capillary bed in the lung mixed venous blood absorbs or excretes PER across the alveolar capillary membrane during and

after exposure respectively and it changes into arterial blood. During breath holding the net gas transfer across the membrane takes place until C_{Alv} tends to equilibrate to C_{ven} —that is, $P_{Alv} = P_{ven}$. During this equilibrium, the net gas transfer is zero and the venous arterial difference is also zero—that is, $P_{ven} = P_{art} = P_{Alv}$. To put it simply: during breath holding, mixed venous blood in the capillary bed absorbs/excretes until the alveolar concentration cannot decrease/increase further. Therefore it is plausible that the decrease or increase in C_{Alv} can be described with an exponential curve.

In our study it is of interest to compare both the measured course (figs 5 and 6) and a theoretical estimated course of alveolar concentration as functions of the residence time during and after exposure; both methods yield the time required (t^*_{eq}) to equilibrate alveolar air with mixed venous blood. To obtain the theoretical course of C_{Alv} a simplified model has been used which is described below and in the appendix.

After each inhalation a certain amount of time is needed before the alveolar concentration reliably represents C_{ven} . The alveolar equilibration time depends upon (a) blood gas partition coefficient (λ), (b) cardiac output ($\dot{Q}$), (c) membrane diffusion capacity, and (d) effective alveolar gas volume (V_{eff}).

PER has a λ value of about 15^{69} and $\dot{Q}$ usually is about 6 l/min. The membrane diffusion capacity (ml gas/min/mm Hg) determines the validity of the common assumption that the partial pressures are similar in both end capillary blood (arterial) and alveolar gas. Once this assumption is seen to be valid, the theoretical approach is simplified.

The transit time of blood along the capillary is in the order of 1 s. In a theoretical study it was shown that the equilibration between C_{Alv} and C_{art} is 99% complete in 0.75 s for gases with high molecular weights (about 200).¹⁰ This figure remains constant even when the membrane diffusion capacity is strongly reduced. Since gases with a high molecular weight represent the greatest delay the assumption is also valid for PER (mol wt: 166).

The size of the effective alveolar volume (V_{eff}) depends on the completeness of mixing of the inhaled air after each inhalation with the functional residual capacity (FRC). When after a single inhalation mixing is instantaneously completed, accurate estimation of C_{ven} by means of exhaled alveolar air requires equilibration of the whole alveolar lung volume (inducing FRC) with mixed venous blood. In 1952, however, Fowler found that in a normal lung after a normal inhalation the gas mixing was uneven.¹¹ During respiration simultaneous convection and gaseous diffusion take place. The latter is gas dependent and appears to be the most important. Nevertheless, the diffusion is clearly not sufficient to achieve

an even ventilation instantaneously for gases with high molecular weights.^{12,13} Gas mixing in the lung may play an important part but a complete description of mixing after a single inhalation would prove complicated. In calculating the theoretical alveolar decrease or increase as functions of residence time during or after exposure, it is assumed that the tidal volume minus the anatomical dead space is instantaneously mixed in the effective alveolar volume (V_{eff}): the latter is already assumed to be in equilibrium with the mixed venous blood. This assumption is justified by the fact that the residence time of the functional residual capacity (FRC) exceeds that of the recently inhaled tidal volume (V_T); this causes a longer equilibration time with mixed venous blood. Our measurements confirm this, since both during and after exposure the concentration of the reserve volume ($t^* \approx 7.0$ s) was of the same magnitude as the C_{Alv} ($t^* = 10$ s).

In fig 7 the alveolar decrease and increase are simulated with different values for V_{eff} , with $\dot{Q} = 61/\text{min}$ and $\lambda = 15$ (eq 1d). The chosen V_{eff} - and $\dot{Q}$ -values are arbitrary. The P_{Ven}/P_I -value—that is, $C_{\text{Alv}}(t^* = 10\text{ s})/C_I$ —has been determined from fig 4 at $t = 14$ minutes. The simulated alveolar curve indicates that during exposure a single inhaled alveolar volume

Fig 7 Schematic alveolar partial pressure relative to venous partial pressure as function of residence time estimated during and after exposure. It is assumed that an inhaled alveolar volume of 0.45 l was mixed during inhalation in effective alveolar volume (V_{eff}), which is already in equilibrium with P_{Ven} . Zero residence time refers to start of inhalation; for simplicity it is assumed that gas exchange takes place after inhalation ($t^* = 2.5$ s).

(0.45 l) mixed in V_{eff} (2–4 l) equilibrates with the mixed venous blood in about 10–16 s. After 10–16 s the simulated P_{Alv} remains constant because P_{Ven} is kept constant. A decrease of λ or $\dot{Q}$ has the same effect on the rate of equilibration as an increase of V_{eff} (eq 1 d).

The results shown in figs 5 and 6 confirm that during exposure the alveolar decrease during the first 10 s of a residence period represents the absorption by mixed venous blood. At $t^* = 10$ s the exhaled alveolar air appears to be representative of the maximal concentration in mixed venous blood for a few seconds. At $t^* > 10$ s the C_{Alv} value also provides a valid estimate of C_{Ven} , but this C_{Ven} value is already influenced by fast kinetic phenomena at the cessation of intake.⁸ Our postexposure results also show that $t^* = 10$ s is sufficient to obtain equilibrium between C_{Alv} and C_{Ven} (fig 4). Therefore, we consider the C_{Alv} ($t^* = 10$ s) as the valid estimate of the PER concentration in mixed venous blood.

During exposure a larger V_{eff} value requires a longer breath holding time. A larger V_{eff} value is caused by more thorough mixing in the lung as well as by deeper inhalation. For fast equilibration with mixed venous blood a small inhaled volume is preferable. From fig 7 it may be seen that breathing conditions during exposure are more critical in respect of residence time than after exposure. This agrees with our results: the residual error of the postexposure results is considerably less than in the results obtained during exposure. Forearm venous blood is usually used as an estimate of mixed venous blood concentrations. Forearm venous blood, however, reflects only a small part of the total and, particularly in the case of fast changes in exposure, it does not reflect local blood coming from remote target organs.⁴ In addition, forearm venous blood may be more influenced by local skin absorption during or up to five hours after exposure.¹⁴ In the postexposure period C_{Art} and C_{Ven} are similar. Particularly in the early postexposure period, however, a forearm venous blood sample cannot replace alveolar air for the estimation of C_{Ven} and the total kinetic behaviour.

MIXED VENOUS-ARTERIAL DIFFERENCE

During and after exposure, each inhalation causes an arteriovenous concentration difference that reaches its maximum almost directly after inhalation. Until the next inhalation this difference decreases by continuing pulmonary absorption/elimination by the blood. In fig 8 the course of P_{Ven} and P_{Art} is shown schematically. If breath holding were continued for a sufficient period ($t^* \approx 10$ s) the difference between P_{Art} and P_{Ven} would approximate zero. P_{Art} fluctuates with each inhalation. During exposure, absorption by blood causes a decrease in P_{Alv} —that is, C_{Alv} —and

Fig 8 Schematic course of alveolar, arterial, and mixed venous partial pressures during and after exposure. During exposure: smooth P_{Ven} course is similar to C_{Alv} ($t^* = 10$ s) as in fig 4. $P_{Alv}(\max)$ is calculated by means of inhaled alveolar volume of 0.45 l with P_1 mixed in $V_{eff} = 2.5$ l (Eq 1c). In practice blood in capillary bed absorbs during inhalation and exhalation; therefore it is difficult to estimate P_{Alv} and P_{Art} at end of inhalation. After exposure, at end of normal exhalation P_{Art}/P_{Ven} ratio equals $C_{Alv}(t^* = 5$ s)/ $C_{Alv}(t^* = 10$ s)-ratio (fig 4).

P_{Art} during exhalation or breath holding. At the beginning of each inhalation P_{Alv} and P_{Art} continue to fall during the first 0.5–1.0 s because the first gas to enter the alveoli is the alveolar gas that filled the dead space at the end of the previous exhalation.¹⁵ After the dead space has been drawn in, P_{Alv} rises sharply; this is because the inhaled volume adds PER far more rapidly than it is absorbed by the blood. If during inhalation absorption by blood were negligible P_{Alv} would increase to an approximated $P_{Alv}(\max)$ —value at $t^* = 2.5$ s (Eq 1c). The magnitude of the fluctuation of P_{Art} can only be approximated roughly: we observed that P_{Alv} ($\approx P_{Art}$) decreased by about 10% during the residence period from 5 to 6 s.

The average P_{Art} and C_{Art} values must be time weighted averages ($\bar{P}_{Art}$, $\bar{C}_{Art}$) rather than simple averages because on entering the capillaries mixed venous blood rapidly absorbs PER during the first fraction of its transit through the capillaries and then progressively more slowly during the later stages of transit. It is assumed that the course of P_{Ven} is smooth, because fluctuations in P_{Art} are damped during perfusion through organs and tissues. The $(P_{Ven} - \bar{P}_{Art})$ or $(C_{Ven} - \bar{C}_{Art})$ differences are expressed as ratios during and after exposure by eqs 2 to 3b (appendix). From eq 3a it follows that at $t = 30$ min the $(\bar{C}_{Art}/C_{Ven})$ ratio equals 1.24. For shorter exposure times this ratio increases; at $t = 1$ min the $(\bar{C}_{Art}/C_{Ven})$

= 1.94. The calculated $(\bar{C}_{Art}/C_{Ven})$ ratios correspond well with the experimental $C_{Alv}(t^* = 5$ s)/ $C_{Alv}(t^* = 10$ s) ratio from the curves in fig 4. This would seem to imply that the concentration in alveolar air after normal zero breath holding ($t^* = 5$ s) is reasonably representative of the time weighted average of the arterial concentration ($\bar{C}_{Art}$). During exposure, however, accurate estimation of the $\bar{C}_{Art}$ value is still arbitrary because of its strong dependence on residence time. After exposure the $(\bar{C}_{Art}/C_{Ven})$ ratio is expected to be constant (eq 2). If again $C_{Alv}(t^* = 5$ s) represents $\bar{C}_{Art}$ and $C_{Alv}(t^* = 10$ s) corresponds to C_{Ven} then this ratio must be a constant with a value of about 0.94. We observed that the ratio ranged from 0.90 to 0.95.

For the later postexposure time our results do not show an increase of the alveolar concentration with increasing t^* for $t^* > 10$ s. These results confirm that $C_{Alv}(t^* = 10$ s) gives the best estimate of PER concentration in mixed venous blood.

To study the kinetic behaviour by means of human exposure experiments, alveolar air sampling can be of great importance for volatile agents and metabolites. A relation was found between the alveolar concentrations of CO_2 and trichloroethane.¹⁶ In and near the factory this relation might be useful to correct afterwards the measured solvent concentration with an accuracy of 10–30%. We think that controlling the residence time is of great importance for accurate

alveolar sampling (<3%) in exposure experiments. Nevertheless outliers can be explained by unusual CO₂ values.

In summary, this study shows that accurate alveolar sampling provides a valid estimate of the PER concentration in mixed venous blood and in arterial blood. Because of the possibility of sampling alveolar air frequently, it provides an opportunity to study the fast and the slow kinetic aspects that become obvious in the mixed venous blood. Interindividual differences in the kinetics will become apparent by alveolar sampling. Mixed venous blood is more directly related to the solvent distribution in the body than arterial blood and therefore it is preferred. The subject inhales normally, holds his breath for 5 s, and exhales normally; at the end of exhalation an alveolar sample is taken of the concentration which seems to provide the best estimate of mixed venous concentration. During exposure, alveolar sampling after longer breath holding gives a valid estimate of C_{Ven}, but this C_{Ven} value is already influenced by fast distribution among fast perfused organs/tissues at stoppage of intake.⁸ An alternative procedure to obtain a valid estimate of mixed venous blood is the concentration in the expiratory reserve volume. The subject inhales and exhales normally; he then exhales a part of the expiratory reserve volume. After normal inhalation and exhalation without breath holding, alveolar concentration provides a reasonable estimate of the time weighted average of the arterial concentration.

The breathing technique for alveolar air sampling is much more critical during exposure than after exposure. During exposure breath holding decreases the alveolar concentration considerably. The alveolar sampling procedure must be described thoroughly when assessing the alveolar concentration or its derivatives such as retention.

In addition, this study shows that in the post-exposure period the accuracy and reproducibility of alveolar sampling, expressed by the small variability in the triplos and small residual errors around a fitted curve is good. This implies that the possible influence of the physiological dead space¹⁶ and ventilation/perfusion imbalances is minimised. The method of sampling, in particular the residence time, must be strictly under control.

Appendix 1

ALVEOLAR MIXED VENOUS EQUILIBRATION

Breath holding

To estimate the time course of C_{Alv} during residence time, it is assumed that the concentration in the end lung capillary blood (arterial) and alveolar concentration C_{Alv} are in equilibrium; this means that C_{Alv} = C_{Art}/λ, with λ = blood/gas partition coefficient. If the

residence time is sufficiently long C_{Alv} equilibrates with the mixed venous concentration, which gives C_{Alv} = C_{Ven}/λ. During residence the blood flow through the capillary bed (Q), C_{Ven} and effective alveolar volume (V_{eff}) are assumed to be constant. The alveolar concentration is a function of the residence time (t*) and follows from a mass balance equation. Expressed by a differential equation:

$$V_{\text{eff}} \left(\frac{dC_{\text{Alv}}}{dt} \right) = (C_{\text{Ven}} - C_{\text{Art}}) Q$$

with C_{Art} = λ · C_{Alv}

The solution for C_{Alv}(t*) both during and after exposure is:

$$C_{\text{Alv}}(t^*) = (C_{\text{Alv}}(t^* \approx 0) - C_{\text{Ven}}/\lambda) e^{-Q\lambda t^*/V_{\text{eff}}} + C_{\text{Ven}}/\lambda \quad (1a)$$

Expressed in corresponding partial pressures

$$P_{\text{Alv}}(t^*) = (P_{\text{Alv}}(t^* \approx 0) - P_{\text{Ven}}) e^{-Q\lambda t^*/V_{\text{eff}}} + P_{\text{Ven}} \quad (1b)$$

The decrease of C_{Alv} started almost directly after the beginning of inhalation with value C_{Alv}(t* ≈ 0) and ends at t* = t*_{eq} with value:

$$C_{\text{Alv}}(t^*_{\text{eq}}) = C_{\text{Ven}}/\lambda \text{ or } P_{\text{Alv}}(t^*_{\text{eq}}) = P_{\text{Ven}}$$

To simplify the estimation of the course of P_{Alv} after an inhalation it is assumed that absorption by mixed venous blood starts at end of inhalation (t* = 2.5 s). The inhaled alveolar volume V_{Alv} with P₁ is mixed in V_{eff} during inhalation.

The starting P_{Alv}(t* = 2.5 s) is estimated as:

$$P_{\text{Alv}}(t^* = 2.5 \text{ s}) = \frac{V_{\text{Alv}} P_1 + (V_{\text{eff}} - V_{\text{Alv}}) P_{\text{Ven}}}{V_{\text{eff}}} \quad (1c)$$

Using this equation with (1b) and dividing by P_{Ven} yields:

$$\frac{P_{\text{Alv}}(t^*)}{P_{\text{Ven}}} = \left[\left(\frac{P_{\text{Ven}}}{P_1} \right)^{-1} - 1 \right] \left(\frac{V_{\text{Alv}}}{V_{\text{eff}}} \right) e^{-Q\lambda t^*/V_{\text{eff}}} + 1 \quad (1d)$$

where t*' = t* - 2.5 s

For calculations of the (P_{Alv}(t*)/P_{Ven}) value, the (P_{Ven}/P₁) value (≈ C(t* = 10 s)/C_i) as a function of the duration of exposure may be determined from fig 4.

Regular breathing

Under regular breathing conditions the concentration in exhaled air is not in equilibrium with C_{Ven} because of the short residence time (t* = 5 s). This means a non-zero (C_{Ven} - C_{Art}) difference. Fahri approached the average (C_{Ven} - C_{Art}) difference in the postexposure period working on two assumptions.¹⁷ The first was that the blood leaving the lung was in equilibrium with the alveolar gas (P_{Alv} = P_{Art} or C_{Alv} = C_{Art}/λ). The second assumption was that the amount of gas

Alveolar sampling of tetrachloroethene

eliminated from the blood into alveolar gas was equal to the amount vented from alveoli into the atmosphere; this would imply that the amount of gas remaining in the alveolar volume was constant (steady state). After exposure under steady state and normal breathing conditions, the following formula could be drawn up:

$$\frac{\bar{C}_{Ar}}{C_{Ven}} = \frac{\bar{P}_{Ar}}{P_{Ven}} = \frac{\lambda \dot{Q}}{\dot{V}_A + \lambda \dot{Q}} \quad (2)$$

where $\dot{V}_A$ = alveolar ventilation

During exposure the reverse process was seen to occur. Working from the same two assumptions the C_{Ar}/C_{Ven} value is now approached by

$$\frac{\bar{C}_{Ar}}{C_{Ven}} = \frac{\lambda \dot{Q}}{\dot{V}_A + \lambda \dot{Q}} + \frac{\lambda \dot{V}_A}{\dot{V}_A + \lambda \dot{Q}} \times \frac{C_I}{C_{Ven}}$$

with $C_{Ven} = C_{Alv}(t^*_{eq})$ the formula is rewritten:

$$\frac{C_{Alv}(t^* = 5s)}{C_{Alv}(t^* = t^*_{eq})} \approx \frac{\bar{C}_{Ar}}{C_{Ven}} = \alpha + (1 - \alpha) C_I/C_{Alv}(t^* = t^*_{eq}) \quad (3a)$$

where:

$$\alpha = \lambda \dot{Q}/(\dot{V}_A + \lambda \dot{Q})$$

In corresponding partial pressures:

$$\frac{P_{Ar}(t^* = 5s)}{P_{Ven}} \approx \frac{\bar{P}_{Ar}}{P_{Ven}} = \alpha + (1 - \alpha) P_I/P_{Ven} \quad (3b)$$

After exposure $\bar{P}_{Ar}/P_{Ven}$ is a constant (Eq 2) whereas during exposure ($\bar{P}_{Ar}/P_{Ven}$) ratio depends on P_I/P_{Ven} .

Appendix 2

SYMBOLS AND ABBREVIATIONS USED IN THE TEXT AND APPENDIX

t^*	Residence time; total time in which gas exchange can take place between inhaled alveolar air and blood in capillary bed of lung (s)
t^*_{eq}	Shortest residence time in which inhaled air equilibrates with mixed venous blood (s)
C_I	Concentration of PER in inhaled air ($\mu\text{g/l}$) during exposure
P_I	Partial pressure of PER in inhaled air (Pa) during exposure
C_{Alv}	Concentration of PER in alveolar air ($\mu\text{g/l}$)
$C_{Alv}(t^* = x s)$	Concentration of PER in alveolar air after a residence time of x seconds ($\mu\text{g/l}$)
C_{Alv}/C_I	Concentration of PER in alveolar air relative to concentration in inhaled air
P_{Alv}	Partial pressure of PER in alveolar air (Pa)

C_{Ven}	Concentration of PER in mixed venous blood returning to lungs ($\mu\text{g/l}$)
P_{Ven}	Partial pressure of PER in mixed venous blood returning to lungs (Pa)
C_{Ar}	Concentration of PER in arterial blood leaving lungs ($\mu\text{g/l}$)
$\bar{C}_{Ar}$	Time weighted average of C_{Ar} ($\mu\text{g/l}$)
P_{Ar}	Partial pressure of PER in arterial blood leaving lungs (Pa)
$\bar{P}_{Ar}$	Time weighted average of P_{Ar} (Pa)
$\dot{Q}$	Cardiac output (l/min)
FRC	Functional residual capacity of lungs (l)
V_{eff}	Effective alveolar volume: alveolar lung volume in which one inhalation causes even ventilation (l)
V_{Alv}	Inhaled alveolar volume (l)
V_T	Tidal volume (l)
MV	Minute volume of ventilation (l/min)
f	Breathing frequency (min^{-1})
$\dot{V}_A$	Alveolar ventilation (l/min)
λ	Blood/air partition coefficient for PER

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- ³ Weinstein L, Swartz MN. Pathogenic properties of invading micro-organisms. In: Sodeman WA Jr, Sodeman WA, eds. *Pathologic physiology: mechanisms of disease*. Philadelphia: W B Saunders, 1974:457-72.