Value of the simultaneous determination of PCO_2 in monitoring exposure to 1,1,1-trichloroethane by breath analysis

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ABSTRACT Eight volunteers were exposed for eight hours to about 200 ppm of 1,1,1-trichloroethane. On the next morning five series of five alveolar samples were collected for the simultaneous determination of PCO_2 and 1,1,1-trichloroethane concentration. Three different methods of sampling were used: voluntary hyperventilation, 10-s breathholding, and "standard." A linear relationship between the alveolar concentrations of both gases was observed in all subjects. Expired air was also collected in two subjects and an analogous relationship was observed. Also the Bohr dead space was found to be of similar size for CO_2 and for 1,1,1-trichloroethane. In the monitoring of solvent exposure by breath analysis it is suggested that the results should be corrected for hyperventilation or hypoventilation and for dilution of alveolar air with dead space air by a proportional adjustment of the solvent concentration at the mean normal alveolar PCO_2 or by disregarding the samples with a PCO_2 outside the normal range. The PCO_2 determination in 40 unselected workers has shown that in more than a third of them, to monitor exposure by breath analysis would have been of little meaning without such an adjustment or rejection criteria.

During the past 20 years, postexposure breath concentrations of various industrial solvents have been studied in volunteers. Biological monitoring of exposed workers was the ultimate aim of these studies. The application in industry of the results obtained from comfortable laboratory conditions, with well-trained volunteers and well-controlled exposures, has been done in a few cases,¹⁻³ but such a method cannot yet be considered as a completely reliable tool until sufficient support has been achieved.

In the 'forties and the 'fifties the pioneers of the physiology of pulmonary gas exchange discussed the pitfalls of alveolar sampling. They have shown that in untrained subjects who have to breathe consciously through a mouthpiece, "truly spontaneous breathing" is extremely difficult⁴ and psychogenic hyperventilation often occurs, which produces a rapid

fall in alveolar PCO_2 .⁵ A reduction of alveolar PCO_2 is also expected if the sampling begins too early during expiration; in this case the alveolar air is contaminated by the "dead space" air which does not take part in the gas exchange. To collect the whole expired air and to use a correction factor for the dead space is not a satisfactory alternative. Indeed, the ratio of the dead space volume to the tidal volume varies with the depth of the respiration and the scatter of individual values is large.⁶ The choice of the alveolar sampling method is also important. Rahn⁷ showed that end-tidal sample, obtained during the course of a normal expiration, yields an alveolar PCO_2 very close to the mean arterial PCO_2 , contrary to the Haldane-Priestley method (sample collected at the end of a forced expiration) which produces too high an alveolar value. Still higher alveolar PCO_2 is obtained with breathholding methods.⁸

It is reasonable to assume that the breath concentrations of a solvent vapour and of CO_2 are affected similarly by hyperventilation, dilution of alveolar air in expired samples, and the method of breath sampling used. Such a relationship was postulated in the design of a few "breath analysers,"

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which have been used for two or three decades and which take into account the amount of alcohol and of CO₂ measured in a breath sample to calculate the expected blood alcohol level.⁹ The reliability of these apparatuses, however, has been challenged by Smith¹⁰ and by Jones *et al.*,¹¹ who have reported that the ratio of alcohol to CO₂ in breath was not constant in all circumstances. On the other hand, the adjustment of breath hydrogen to a constant alveolar concentration of CO₂ or O₂ has been shown to improve the reproducibility of H₂ levels in the study of gastrointestinal disorders in man.^{12 13}

This study examines the usefulness of the PCO₂ determination in the monitoring of solvent exposure by breath analysis. 1,1,1-trichloroethane has been chosen because of its slow metabolic rate and its high lipid solubility, which leads to sufficient storage in the body for subsequent breath analysis one day after exposure. At this time the decay of alveolar concentration is very slow and can be neglected within a period of a few hours.

Methods

Eight male volunteers, staff members and medical students, were exposed, at rest, from 8 to 12 am and from 1 to 5 pm, with a ten-minute break each time, to a mean concentration of 1,1,1-trichloroethane ranging from 203 to 236 ppm (table 1).

Table 1 Age, weight, and height of the eight volunteers and mean exposure concentration of 1,1,1-trichloroethane

Subject	Age (y)	Weight (kg)	Height (m)	Mean exposure (ppm)
1	27	58	1.64	225
2	26	64	1.71	236
3	47	75	1.76	203
4	36	65	1.73	203
5	21	64	1.71	205
6	22	63	1.73	205
7	21	80	1.83	213
8	24	73	1.76	213

The experimentation chamber and the method used to obtain the desired concentrations in the air have been described elsewhere.¹⁴

Alveolar sampling was carried out on the next day between 8 and 12 am, and three sampling methods were tested. *Method 1* ("standard"): the sample was collected during the last part of a prolonged (but not forced) expiration following a normal inspiration. *Method 2* (hyperventilation): the sample was taken at the end of a forced expiration after 15 seconds of voluntary deep and rapid respiration. *Method 3* (breathholding): the sample was also collected at the end of a forced expiration but after ten seconds of breathholding. This rather

short breathholding time was chosen because it was supposed to be the most convenient for later application in industry. With each method the subject was sitting, and sampling was done after a period of quiet breathing through the sampling valve for one or two minutes. The sampling schedule included five series, at about one-hour intervals, of five consecutive samples in random order (three standard test and one with each of the other two methods of sampling). In subjects 1 and 2 expired air was collected for each series of sampling during the expiration. This was done for all samples in subject 2 and only for the standard tests in subject 1, at the same expiration as the one used for analysis of alveolar air.

Forty unselected workers from several industries, who came to the institute at various dates for a routine medical examination, were asked whether they would agree to undergo a few breathing tests and none refused. After a standardised explanation, four alveolar samples were collected for PCO₂ determination, out of which three samples were carried out with the standard method and one with the breathholding one.

ANALYSES

Alveolar air was sampled into a one-way valve made of glass and heated to about 40°C with a heating coil (fig 1) to avoid water condensation. In such a valve air is continuously aspirated from the small outlet in the "expired air" part of the valve and passes through one cell (<1 ml) for the infrared analysis of CO₂ and then into two sample loops (5 ml each) of a dual columns gas chromatograph. The whole tubing is heated above 40°C. The dead space between the valve and the IR cell is under 10 ml, and between the IR cell and the GC loops under 1.5 ml. These dead spaces correspond to a negligible delay in the reading of CO₂ since the sampling pump of the system (Beckman LB2, see below) is operated at about 0.5 l/min (>8.3 ml/s). An electric valve placed between the pump and the sampling loops allowed the aspiration to be interrupted in order to inject the chosen gas samples into the GC. Expired air was collected in heated plastic bags (Saran) of a volume of about 2 litres and analysed for 1,1,1-trichloroethane and CO₂ concentrations shortly (2 to 15 minutes) after sampling.

1,1,1-trichloroethane was analysed by gas chromatography under the following conditions:

- Glass column filled with Apiezon L,
60°C
2 metres long
2 mm diam.
15% on Chromosorb W
280-100 mesh (~50 µm)

Fig 1 Sampling valve for breath analysis.

—Flame ionisation detector and N_2 flow of about 20 ml/min

—Instrument: Perkin Elmer 900

For volunteers 1 and 2, the analysis of CO_2 was performed by gas chromatography on silicagel, 20-40 mesh at $60^\circ C$, 1 m long, 2 mm diameter and with "hot wire" detection and helium as carrier gas. For the other volunteers and the workers, CO_2 was measured continuously with a Beckman Medical Gas Analyzer LB2. This continuous visualisation of the CO_2 concentration was helpful in obtaining the sample of alveolar air when the level was at its "plateau," by stopping the air flow with the system described above.

Calibration was carried out for 1,1,1-trichloroethane (purris pa Fluka, Switzerland) with known concentration prepared statically in a glass container and for CO_2 with calibrated gas 10.0% purchased from Schweissttechnik AG, Switzerland.

Air temperature, humidity, and pressure were continuously measured with a thermohygrograph Type Haenni, Haenni, Switzerland.

Partial pressure of CO_2 (in kPa) was corrected to

BTPS conditions according to the following formula:

$$P_{CO_2} = \frac{A (PB - 6.3)}{100 (PB - 5.5)} \times 101.3$$

Where A: CO_2 concentration in breath in percent volume (%)

PB: Barometric pressure of the day of the experiment in kPa

6.3: Vapour pressure of H_2O at $37^\circ C$ in kPa (= 47 mmHg)

5.5: Vapour pressure of H_2O at $34.5^\circ C$ (temperature at the mouth¹⁵) in kPa (= 41 mmHg)

101.3: Reference pressure in kPa (760 mmHg).

The observed alveolar concentration of 1,1,1-trichloroethane (COBS) was corrected (CCORR) by a simple proportion adjustment at a P_{CO_2} of 5.1 kPa

$$^{CCORR} = ^{COBS} \times \frac{5.1}{P_{CO_2OBS}}$$

5.1 kPa (38 mmHg) was chosen because it is the mean normal value of P_{CO_2} at a height of about 500 m.¹⁶

Two alveolar samples gave incoherent results due to technical faults such as incorrect handling of valves, leakages, or time shift between the attainment of the plateau and injection into the gas chromatograph and were disregarded. There were one such sample for subjects 7 and 8. For the expired air samples a few had to be eliminated due to difficulties in handling the plastic bags (non-uniform heating leading to condensation and leakage). There were three such samples for volunteers 1 and 2.

Results

Figure 2 shows 1,1,1-trichloroethane concentration plotted against P_{CO_2} for all the alveolar samples collected between 8 and 12 am the day after the exposure in five series of five consecutive tests. A linear relationship between the concentrations of both gases is observed in the eight subjects. Voluntary hyperventilation produces a fall of P_{CO_2} to about half its normal level, which is paralleled by a similar decrease of the solvent concentration. In subjects 1 and 2 some samples collected with the standard or the breathholding method of sampling yield a P_{CO_2} value below the normal range of 4.7 to 6.1 kPa (35 to 46 mmHg),¹⁷ which may be explained by unconscious hyperventilation. Conversely, the P_{CO_2} values above normal observed in subjects 5, 6, and 7 are probably due to a transient slight hypoventilation. In both cases the relative variation of 1,1,1-trichloroethane concentration is similar to that of P_{CO_2} .

Fig 2 1,1,1-trichloroethane concentration (ppm) against PCO_2 (kPa) in alveolar air for eight volunteers. Between dotted lines is range of normal PCO_2 : 4.7-6.1 kPa. (• = standard method of sampling, × = 10-s breathholding, Δ = voluntary hyperventilation).

Comparison of the results of the eight volunteers shows large differences among the subjects in the level of 1,1,1-trichloroethane concentrations, which is about twice as high in subjects 1, 3, and 4 as in

subjects 5 and 8 (fig 2). These differences explain the varied slopes of the regression lines which all have an intercept on the y axis close to zero. The intercept, however, is always slightly negative (for a

theoretical PCO_2 value of 0, the solvent concentration becomes negative). This might be due to a slightly different behaviour of CO_2 and 1,1,1-trichloroethane when passing from the blood to the alveoli or from the latter to the mouth, or both. Nevertheless, this minimal difference is negligible, and a direct proportionality between PCO_2 and the solvent concentration can be postulated.

If hyperventilation or hypoventilation occurs during alveolar sampling of 1,1,1-trichloroethane it can therefore be suggested that the observed concentration is corrected by a proportional adjustment at the mean normal value of PCO_2 , that is 5.1 kPa (38 mmHg) at our altitude¹⁶ (see: methods). The corrected concentration of the solvent will thus correspond to the one which would have been measured in conditions of "isoventilation."

Expired air was also collected in subjects 1 and 2, and the results may be seen in fig 3. Owing to the

Fig 3 1,1,1-trichloroethane concentration (ppm) against PCO_2 (kPa) in expired air for volunteers 1 and 2. (• = standard method of sampling, × = 10-s breathholding, △ = voluntary hyperventilation).

dilution of alveolar air with the air of the dead space, the gas concentrations are of course lower than those found in the alveolar samples for the same subjects (fig 2). The linear relationship between PCO_2 and 1,1,1-trichloroethane concentration, however, is as strong in expired air as in alveolar air. This raises the question whether the solvent concentration could also be corrected in the case of dilution with the dead space air. Such a correction assumes that the Bohr dead space is of similar size for CO_2 and for 1,1,1-trichloroethane. Since the alveolar samples were collected at the same expirations as the ones used for the determination of expired gas concentrations, this assumption can be verified. As shown (fig 4) the dead space is similar for both gases, and the adjustment of the solvent

Fig 4 Ratio of Bohr dead space to tidal volume (V_D/V_E) for 1,1,1-trichloroethane versus V_D/V_E ratio for CO_2 . According to the Bohr equation¹⁷: $V_D/V_E = (F_A - F_E)/F_A$ where F_A and F_E are fractional concentrations in alveolar gas and in expired gas, respectively. (• = standard method of sampling, × = 10-s breathholding, △ = voluntary hyperventilation).

concentration at a PCO_2 of 5.1 kPa is therefore applicable to the analysis of expired air or of alveolar samples contaminated by the dead space air.

Table 2 shows the average of the corrected 1,1,1-trichloroethane alveolar concentrations for the five series of tests of the eight volunteers. In most subjects a slight decrease is observed between 8 and 12 am, which is in accordance with the theoretical decay curve calculated for a similar solvent.¹⁸ The difference between subjects of the mean alveolar 1,1,1-trichloroethane concentration pointed out before is unrelated to the exposure which was similar (table 1); it can be explained by different retention rates of the solvent. The main determinants of solvent retention, about one day after exposure, are size of the body fat depot and the ventilation rate since exposure.¹⁸ The former is positively related to the retention rate and the latter, which depends on physical activity, negatively.

The result of the measurements of alveolar PCO_2 in 40 unselected workers is shown by the histogram (fig 5). About one-third of the PCO_2 values are below the normal range, which could be due to emotional hyperventilation or to dilution with the dead space air, or both. In addition, a few PCO_2 values are above the normal range perhaps because the subjects overcorrect, in their effort to obtain a "good" sample, the natural tendency to hyperventilate when breathing consciously. Furthermore, three out of 40 workers were unable to give alveolar air by the standard method, and two by the breath-holding one.

Table 2 Mean corrected 1,1,1-trichloroethane alveolar concentration ($\pm$ SD) between 8 to 12 am one day after an exposure of eight hours to about 200 ppm. Each value corresponds to an average of five successive tests obtained from alveolar air (a) or from expired air (b). The observed concentration of 1,1,1-trichloroethane has been adjusted at a PCO_2 of 5.1 kPa (38 mmHg)

Subject	Approximate time (h)				
	8	9	10	11	12
1 (a)	7.5 $\pm$ 0.4	7.4 $\pm$ 0.6	7.0 $\pm$ 0.3	6.4 $\pm$ 0.3	8.7 $\pm$ 0.2
(b)*	7.1 $\pm$ 0.3	7.2 $\pm$ 0.2	6.8 $\pm$ 0.5	5.9 $\pm$ 0.2	9.0†
2 (a)	5.6 $\pm$ 0.9	5.4 $\pm$ 0.3	5.0 $\pm$ 0.2	4.6 $\pm$ 0.3	4.6 $\pm$ 0.1
(b)	5.4 $\pm$ 0.3†	5.3 $\pm$ 0.2	4.7 $\pm$ 0.1	4.6 $\pm$ 0.3	4.5 $\pm$ 0.2
3 (a)	5.1 $\pm$ 0.5	5.2 $\pm$ 0.5	5.5 $\pm$ 0.3	5.1 $\pm$ 0.5	5.5 $\pm$ 0.1
4 (a)	5.6 $\pm$ 0.3	5.3 $\pm$ 0.3	5.3 $\pm$ 0.3	6.6 $\pm$ 0.3	4.7 $\pm$ 0.8
5 (a)	3.1 $\pm$ 0.2	3.2 $\pm$ 0.3	3.1 $\pm$ 0.3	2.9 $\pm$ 0.1	2.8 $\pm$ 0.2
6 (a)	3.8 $\pm$ 0.3	3.7 $\pm$ 0.0	3.7 $\pm$ 0.1	3.6 $\pm$ 0.2	3.3 $\pm$ 0.1
7 (a)	5.1 $\pm$ 0.3	4.4 $\pm$ 0.1	3.9 $\pm$ 0.4	3.4 $\pm$ 0.4	3.7 $\pm$ 0.3
8 (a)	3.9 $\pm$ 0.2	3.6 $\pm$ 0.3	3.4 $\pm$ 0.3	3.0 $\pm$ 0.1	2.9 $\pm$ 0.1

*Average of only the three tests with standard method of sampling.

†Two samples out of five failed.

‡Two samples out of three failed.

Fig 5 Histogram of PCO_2 (kPa) measured in 40 unselected workers with standard method of sampling (mean of three tests) and breathholding method (one test). Three workers failed with standard method and two with breathholding one.

Discussion

The pitfalls of the biological monitoring of exposure to industrial solvents by breath analysis are numerous. A practicable method to collect the breath samples in or near the factory, to analyse them on the spot or to keep their content unaltered until later analysis in a well-equipped laboratory, has not yet been fully

developed. Without speaking of the interpretation of the results in cases of fluctuating or intermittent exposure, the suitable time of sampling is very difficult to choose. Sampling shortly after exposure is not satisfactory, since the drop of the solvent concentration in alveolar air is too fast,¹⁸ and it is unrealistic to expect the workers to stay on for a long time at the end of the working day. The results of sampling on the next morning may be difficult to relate to the mean exposure of the preceding day because, as we have seen, the solvent concentration is then strongly influenced by body fat and activity since exposure. In sampling inside the workshop towards the end of exposure it seems difficult to avoid contamination of the sample by ambient air, in which the solvent concentration is much higher than in the breath of the exposed workers.

In this paper we have dealt only with the preliminary condition of controlling breath sampling for emotional hyperventilation, transient hypoventilation, and dilution of alveolar air with dead space air, which were shown to occur in a significant proportion of 40 unselected workers. We have suggested a proportional adjustment of the 1,1,1-trichloroethane concentration at a PCO_2 of 5.1 kPa (38 mmHg). The strength of the relationship found in eight volunteers between the gas concentrations, its independence from the amount of solvent retained, and the similarity of the Bohr dead space for CO_2 and for the solvent, support the use of such an adjustment. An analogy can be drawn between this PCO_2 correction and the creatinine correction of metabolites concentration in a urine sample.

Alternatively, in waiting for confirmation of our results in further studies on this and other industrial solvents, the alveolar samples with a PCO_2 outside the normal range should be disregarded.

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Appendix

Table 2 shows the mean of corrected alveolar concentration of 1,1,1-trichloroethane for the five series of tests (3 standard, 1 hyperventilation and 1 breathholding tests in each series).

A variance analysis¹⁹ was carried out on the corrected and uncorrected (observed) individual results obtained with the standard method (3 tests in each series). The usual following scheme was obtained.

ANALYSIS OF VARIANCE

Source of variation	Degree of freedom	Sum of squares	Mean square	F or variance ratio
Between series	4	B	B/4	B/4
Within series	10	A-B	(A-B)/10	(A-B)/10
Total	14	A		

$$\text{Where } A = \sum_{i=1}^5 \sum_{j=1}^3 \times_{ij}^2 - \left(\sum_{i=1}^5 \sum_{j=1}^3 \times_i \right)^2 / 15$$

i = series

j = tests or measurement

ij denotes the jth measurement of the ith series

$$B = \sum_{i=1}^5 \left(\sum_{j=1}^3 \times_i \right)^2 / 3 - \left(\sum_{i=1}^5 \sum_{j=1}^3 \times_i \right)^2 / 15$$

The coefficient of variation (CV), which is simply the ratio of the within standard error $\left(\sqrt{\frac{A-B}{10}} \right)$ to the overall mean of all the samples $\left(\sum_{i=1}^5 \sum_{j=1}^3 \times_i / 15 \right)$,

expressed in percentage, represents the reproducibility of one single measurement of alveolar concentration. It could be considered as quite good when it lies below 5% and "fair" between 5 and 10%.

The results of series variance analysis are summarised in table 3. The F ratio is significant at $p < 0.001$ in 12 out of the 16 cases, which means that the differences observed between the series of tests can be considered as significant.

The coefficient of variation varies from 2.0 to 12.0% with an average of 5.6%, which is satisfying. The correction of the values does not improve systematically the reproducibility, which tends to

Table 3 Results of variance analysis carried out from the observed (a) or corrected (b) results on the five series of three "standard" tests for each subject

Subject	F ratio	p	CV (%)
1 (a)	23.6	< 0.001	8.6
(b)	12.6	< 0.001	5.0
2 (a)	2.0	NS	12.0
(b)	1.6	NS	10.3
3 (a)	0.8	NS	5.9
(b)	1.1	NS	6.1
4 (a)	9.4	< 0.001	6.7
(b)	8.9	< 0.001	6.1
5 (a)	20.2	< 0.001	2.7
(b)	13.8	< 0.001	2.7
6 (a)	28.7	< 0.001	4.2
(b)	21.1	< 0.001	2.0
7 (a)	32.1	< 0.001	4.5
(b)	19.1	< 0.001	5.9
8 (a)	87.5	< 0.001	2.7
(b)	33.3	< 0.001	3.9

CV = Coefficient of variation.

NS = Not significant.

show that the major source of variation is probably more an analytical one than a fluctuation of the subject's ventilation.

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