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Respiratory input in inhalation experiments

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ABSTRACT The definition of the respiratory input in experimental human exposure to volatile solvents was examined on theoretical grounds. The respiratory rate of input may be defined as the rate of uptake that equals the inhaled minus exhaled amount per minute. In the present paper the rate of respiratory input is defined as the rate of the functional intake (RFI) which equals the product of the inhaled concentration (C_I) and a functional alveolar ventilation ($\dot{V}_a$). The functional $\dot{V}_a$ is a virtual alveolar volume per minute which equilibrates completely with the mixed venous blood. Human subjects were exposed simultaneously to tetrachloroethene (PER, perchloroethylene) and trichloroethene (TRI) in order to study the consequences of the application of both definitions. It is shown that when using the uptake as the respiratory input some misleading conclusions may be drawn on (*a*) the dependance of the metabolised fraction on the duration of exposure, (*b*) the dependance of the kinetic characteristic on the duration and route of administration, and (*c*) the changes of the rate of metabolism during exposure due to physical exercise. The respiratory input defined as the rate of functional intake (RFI) rejects these misleading conclusions.

In human exposure experiments the rate of respiratory input of solvents during exposure has been usually defined as the rate of uptake which equals the inhaled minus the exhaled amount per minute.[1–6] At first sight this definition seems obvious because the retained amount in the body during exposure seems to be equivalent to the dose used in pharmaceutics.

In the present study the rate of respiratory input has been defined as a rate of the functional intake (RFI). The basic idea is that exhalation of the solvent is a continuous process that occurs both during and after exposure. In fact, during exposure the input and pulmonary excretion occur simultaneously. This is in analogy with oral and intravenous administration.

The purpose of the study was to examine whether, on theoretical grounds, the choice of the input definition affects the conclusions on the exhaled fraction and thus on the metabolised fraction and in addition on the kinetic response.

Subjects were exposed two or three times simultaneously to vapour of PER and TRI during rest, 30 W, and 65 W physical exercise. For both PER and TRI the concentration in alveolar air was measured during and after exposure. The consequences of the use of both input definitions for the metabolised fraction will be studied.

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This study is part of an extended study for the application of a method that permits the retrospective estimation of the RFI of an individual.[7] This method requires knowledge of the individual kinetic response. In addition the intraindividual and interindividual variability of kinetics of PER and TRI have also been studied (J J G Opdam, in preparation).

Theoretical background

RESPIRATORY INPUT

In exposure experiments the kinetic response—that is, the concentration/time curve—may be expressed by a mathematical function with coefficients c(i) and exponents r(i) (appendix 1).

The experimental determination of this function should not be affected by the route or duration of administration. This means that for a solvent, an intravenous infusion and a respiratory exposure should both yield a kinetic response with the same coefficients c(i) and exponents r(i). Therefore, as in the case of intravenous infusion, pulmonary excretion is assumed to occur both during and after exposure.

To define the rate of respiratory input a simple approach has been applied (fig 1). The lungs serve as a tool to transport the vapour between ambient air and blood; during and after exposure the body responds with, respectively, an increase and a decrease of the concentration in mixed venous blood (C_{ven}) (fig 2). It

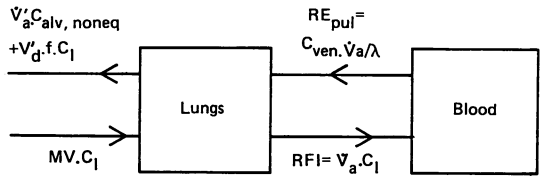

Fig 1 *Vapour transport between lungs and blood as simple model. MV: minute volume of ventilation; f: respiratory frequency, and C_I: inhaled concentration. (Further abbreviations are given in appendix 2.)*

has previously been shown for tetrachloroethene that during exposure the alveolar concentration after a normal inhalation and exhalation had not come into equilibrium with C_{ven}.[8] After five seconds breath-holding, however, the alveolar concentration did equilibrate with C_{ven}. The non-equilibrated alveolar concentration ($C_{alv,\,noneq}$) exceeds the equilibrated alveolar concentration ($C_{alv,\,eq}$). Thus under conditions of normal respiration $C_{alv,\,noneq} > C_{alv,\,eq}$: area A3 is not zero (fig 2). During exposure the absorption into and the excretion out of the blood are considered to be simultaneous processes. Pulmonary excretion occurs during and after exposure and the rate of pulmonary excretion (RE_{pul}) is set to be proportional to C_{ven} with the pulmonary clearance $\dot{V}_a/\lambda$ as the proportional constant:

$$RE_{pul} = \dot{V}_a/\lambda.\, C_{ven} = \dot{V}_a.\, C_{alv,\,eq} \qquad (1a)$$

with blood/air partition coefficient λ and $C_{ven}/\lambda = C_{alv,\,eq}$.

The rate of input is defined as:

$$RFI = C_I.\, \dot{V}_a \qquad (1b)$$

The $\dot{V}_a$ is the functional alveolar ventilation and has

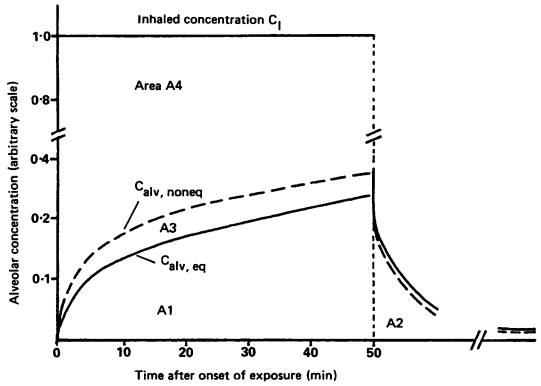

Fig 2 *Schematic time courses during and after exposure of inhaled concentration C_I and alveolar concentrations $C_{alv,\,noneq}$ and $C_{alv,\,eq}$. A1 and A2 present areas under C_{alv} in equilibrium with C_{ven} ($= \lambda\, C_{alv,\,eq}$). A3 and A4 present areas between concentrations.*

been defined as a virtual alveolar air volume per minute in which the solvent equilibrates completely with that in the blood. A relatively low $\dot{V}_a$ corresponds to a large functional (physiological) dead volume (V_d), whereas a relatively large $\dot{V}_a$ corresponds to a small V_d. The total V_d is the sum of the anatomical – ($V'_d \simeq$ 0·15 l) and the alveolar dead volume.

The subject inhales an amount of solvent per minute which equals $MV.C_I$ from which a part (RFI) will be absorbed by the blood (fig 1). This part depends only on $\dot{V}_a$ and C_I (eq 1b), whereas the pulmonary excretion depends only on $\dot{V}_a/\lambda$ and C_{ven}. When, under conditions of normal respiration, all the solvent in the anatomical alveolar volume (V'_a) equilibrates instantaneously with that in the blood, then the functional alveolar volume (V_a) becomes maximal; $V_a = V'_a$ and $\dot{V}_a = \dot{V}'_a$; in this case $C_{alv,\,noneq} = C_{alv,\,eq}$ and area A3 is zero. On the other hand, when there is no diffusion across the membranes V_a and, therefore, $\dot{V}_a$ and RFI are zero; $C_{alv,\,noneq} = C_I$: A3 is maximal.

Under normal respiratory conditions $\dot{V}_a$ will be smaller than $\dot{V}'_a$, A3 is not zero. $\dot{V}_a$ depends on the agent. For example, solvents with a low blood/air partitition coefficient λ will slowly equilibrate with the blood and, therefore, under normal respiratory conditions $\dot{V}_a$ will be relatively small. In this case area A3 is relatively large. In fact, a large area A3 relative to area A3 + A4 corresponds to a large functional dead space.

It is worth while keeping in mind that $C_{alv,\,noneq}$ is composed of the functional volume V_a with $C_{alv,\,eq}$ and a volume $V'_a - V_a$ with a concentration between C_I and $C_{alv,\,eq}$. Therefore $C_{alv,\,noneq}$ does not indicate the RE_{pul} (eq 1a).

It is assumed that during a short period the amount of solvent in the lungs is a constant which means the input = output (fig 1) thus with eqs 1a and 1b:

$$MV.\,(C_I - C_e) = \dot{V}_a.\,(C_I - C_{alv,\,eq}) \qquad (2)$$

with: $$MV = \dot{V}'_a + f.\,V'_d \qquad (3)$$

$$C_e = (\dot{V}'_a.\,C_{alv,\,noneq} + f.\,V'_d.\,C_I)/MV \qquad (4)$$

When the values of $\dot{V}_a$ and $\dot{V}'_a$ are taken during the period of exposure t_1 the equations 2, 3, and 4 yield the ratio:

$$\frac{\dot{V}_a}{\dot{V}'_a} = \frac{C_I.\,t_1 - AREA\,(C_{alv,noneq})}{C_I.\,t_1 - AREA\,(C_{alv,eq})} \qquad (5)$$

where the numerator equals area A4 and the denominator equals area (A3 + A4) in fig 2. This ratio $\leqslant 1$ as $C_{alv,\,noneq} \geqslant C_{alv,\,eq}$.

The functional $\dot{V}_a$ during exposure may be estimated with use of the total amount (U_{tot}) retained in the body. The left side of equation 2 represents the retained amount per minute. The average $\dot{V}_a$ may be estimated with:

$$\dot{V}_a = U_{tot}/(C_I.\,t_1 - A1) \qquad (6)$$

In the present paper the RFI according to eq 1b is taken as the rate of respiratory input, whereas the usual definition of the input in inhalation experiments equals the rate of uptake ($\dot{U}$) and is given by the left side of eq 2:

$$\dot{U} = MV.(C_I - C_e) \quad (7)$$

EXHALED FRACTION

The exhaled amount is usually calculated by the product of the minute volume of ventilation (MV) and the exhaled concentration (C_e). In functional terms this means the functional alveolar ventilation ($\dot{V}_a$) and the alveolar concentration in equilibrium with mixed venous blood ($C_{alv,eq}$): the rate of exhaled amount equals $\dot{V}_a \times C_{alv,eq}$ (eq 1a). During and after exposure the exhaled amounts relative to the functional intake equal the fractions F_{i1} and F_{i2} in eqs 8a and 8b respectively:

$$F_{i1} = \dot{V}_a.\,A1/RFI.\,t_1 = A1/C_I.t_1 \quad (8a)$$

$$F_{i2} = \dot{V}_a^*.\,A2/RFI.\,t_1 \quad (8b)$$

The fraction F_{i1} equals the time weighted $C_{alv,eq}$ divided by C_I. The total exhaled amount during and after exposure relative to the functional intake equals the fraction F_i:

$$F_i = F_{i1} + F_{i2} \quad (9)$$

$\dot{V}_a$ and $\dot{V}_a^*$ are the functional alveolar ventilations during and after exposure respectively (fig 2).

For both TRI and PER the $\dot{V}_a$ during exposure may be obtained experimentally according to eq 6. The areas A1 and A2 are the areas under the $C_{alv,eq}$ curves (fig 2) and may be obtained with analytical integration of the $C_{alv,eq}$ curves (appendix 1). The amount U_{tot} may be determined as described below.

In the postexposure period the $\dot{V}_a^*$ is unknown. With the assumption that PER is not bound and completely exhaled it is easy to see that for PER the average value of $\dot{V}_a^*$ may be approximated by:

$$V_a^* = U_{tot}/A2 \quad (10)$$

To determine the postexposure $\dot{V}_a^*$ of TRI this $\dot{V}_a^*$ has been set equal to the $\dot{V}_a^*$ of PER. That the $\dot{V}_a$ values for TRI and PER are nearly the same will be shown below. In fact, because of the simultaneous exposure to the non-inert TRI and the inert PER, the data of PER may then be used as an internal standard for the non-inert agent. The exhaled amount relative to the uptake equals:

$$F_u = A2.\,\dot{V}_a^*/\dot{U}.\,t_1 \quad (11)$$

RETENTION

In functional terms the functional alveolar retention (R_{alv}) may be defined as

$$R_{alv} = 1 - C_{alv,eq}/C_I \quad (12)$$

The exhaled alveolar air is in equilibrium with C_{ven}.

Table 1 *The minute volume of ventilation (MV) and the respiratory frequency (f) for four subjects exposed to PER under conditions of rest*

	Rest 1		*Rest 2*	
Subject	*MV (l/min)*	*f (/min)*	*MV (l/min)*	*f (/min)*
1 m	6·7	12	6·6	12
2 m	7·5	11	7·0	11
1 f	5·4	7	5·1	8
2 f	4·8	9	4·8	8

The rate of uptake can be calculated as

$$\dot{U} = R_{alv}.\,RFI \quad (13)$$

The usual definition of the retention equals

$$R = 1 - C_e/C_I \quad (14)$$

By contrast with R_{alv} the retention R is not a good indicator of the uptake. At a constant rate of uptake, C_e and consequently R may vary due to variations in, for example, the respiratory frequency.[9]

The rate of uptake may be calculated as

$$\dot{U} = MV.\,R.\,C_I \quad (15)$$

in which MV.R equals the lung clearance.[9]

Methods

EXPOSURE PROCEDURE

In a first set of experiments four subjects (2 men, 2 women) were exposed twice at rest to tetrachloroethene (PER). A second set of experiments was carried out with six subjects (3 men, 3 women); each subject was exposed three times simultaneously to tetrachloroethene and trichloroethene (TRI) at rest, 30 W, and 65 W physical activity. The minute volume of ventilation (MV) and respiratory frequency (f) are presented in tables 1 and 2.

Concentrations in inhaled air ranged from 0·11 to 0·43 μmol/l (18–71 μg/l) for PER and from 0·24 to 1·58 μmol/l (31–205 μg/l) for TRI. Periods of exposure ranged from 29 to 62 minutes. Before, during, and the first 10 minutes after exposure the subject was in a

Table 2 *The minute volume of ventilation (MV) and the respiratory frequency (f) for six subjects exposed simultaneously to PER and TRI under conditions of rest, 30 W, and 65 W physical exercise*

	Rest		*30 W*		*65 W*	
Subject	*MV (l/min)*	*f (/min)*	*MV (l/min)*	*f (/min)*	*MV (l/min)*	*f (/min)*
1 m	5·1	9	17·7	14	24·6	12
3 m	6·0	8	16·3	15	21·2	11
4 m	8·7	10	16·4	12	21·4	13
3 f	7·7	12	16·9	13	29·5	20
4 f	5·4	11	15·1	16	20·2	20
5 f	9·9	16	16·2	21	25·5	23

sitting position and breathed continuously through a valve. During exposure the valve was connected with a Tedlar-Bag. This bag contained vapour of PER and TRI and at the end of exposure the bag was shut off and the subject immediately inhaled fresh air.

The experiments with physical exercise during exposure were carried out with a bicycle ergometer. The subject started cycling 10 minutes before the exposure and stopped just at the end of exposure; cycling was carried out with 70 ± 5 cycles/min.

During exposure two different alveolar air sampling methods were used: method 1: normal inhalation followed by normal exhalation; method 2: normal inhalation, breathholding for five seconds followed by normal exhalation. For both methods the last part of the normal exhalation was collected. During exposure the alveolar air was sampled about seven times with method 1 and 12 times with method 2. Methods 1 and 2 yield the alveolar concentrations $C_{alv,\,noneq}$ and $C_{alv,\,eq}$ respectively (fig 2). The latter, measured after five seconds of breath holding may be considered as the alveolar concentration in equilibrium with that in mixed venous blood.[8]

The postexposure period of observation lasted 70–500 hours for PER and 20–320 hours for TRI. This period was mostly limited by the analytical detection level or a background level in ambient air. The subjects were asked not to drink alcohol and not to sport intensely during the first three postexposure days. Beyond this period the subjects followed their normal daily life style.

In the first 30 minutes after exposure alveolar air samples by means of method 2 were carried out each for two to three minutes. Then alveolar air sampling according to method 2 was carried out by the subject himself in duplo each 20 minutes; after two hours the subject sampled each hour. During the following days the subject sampled his alveolar air according to method 2 in triplo just before going to sleep and next morning immediately after awakening. In addition ambient air at home was sampled frequently by the subject.

APPARATUS AND GAS CHROMATOGRAPHIC ANALYSIS

The method of exposure and of collecting alveolar air has been described previously.[8] Alveolar air samples were collected with the aid of vacuum sampling tubes that were plugged into a portable glass tube system; the system was heated to 37°C.

The inhaled air with concentrations of PER and TRI came through a Tedlar-Bag; the bag served as a buffer and was filled continuously with 60% H_2O saturated air, PER and TRI coming from three gas bombs.

The method of sampling at home was similar to that in the laboratory. The vacuum sampling tubes and a short tube system were heated to 37°C by means of a small portable stove.

The bicycle ergometer (Lode Instruments, The Netherlands) is a constant power bicycle ergometer—that is, the power delivered does not depend on the cycle frequency.

The analyses were carried out by gas chromatography.[8] With a gas tight syringe (Hamilton) a volume of 25 or 250 μl was injected. The 250 μl was injected for samples of the late postexposure period in which the concentration was very low. The detection limit with a 25 μl injection volume was about 0·01 μmol/m^3 (1 μg/m^3) for PER and 0·03 μmol/m^3 (4 μg/m^3) for TRI. The background level in ambient air was about 0·1 μmol/m^3 for PER whereas for TRI it was below the detection limit.

KINETIC RESPONSE

The kinetic response—that is, the $C_{alv,eq}$ curve during and after exposure—is expressed as a sum of exponential terms (appendix 1 eqs 1 and 2).

The coefficients c(i) and exponents r(i) in these equations were estimated by means of curve fitting. The following $C_{alv,eq}$ data were used: (1) the level of $C_{alv,eq}$ during exposure just before the end of exposure; (2) all $C_{alv,eq}$ values during the first postexposure day, and (3) all $C_{alv,eq}$ values sampled at the subsequent mornings. The level of $C_{alv,eq}$ at the end of exposure was used to limit the fitted $C_{alv,eq}$ level at the end of exposure. The $C_{alv,eq}$ data in the morning rather than the C_{alv} in the evening were used because of the previous "standardised" sleeping period. In this way the impact on C_{alv} of external factors such as physical activity has been minimised.

The $C_{alv,eq}$ data were fitted by means of minimising the sum of weighted least squares. The fitting was carried out with a sequential simplex procedure[10] which seems to be preferred for biological data.[11]

RATE OF INPUT

The RFI was determined according to equations 1b and 6. The total retained amount (U_{tot}) of solvent during exposure was determined by means of the difference between the inhaled and exhaled amounts. The total exhaled amount of solvent was collected in Tedlar-Bags. The total inhaled amount was determined as the product of the inhaled concentration C_I and the inhaled volume. To determine C_I the inhaled air was sampled about six times. The inhaled volume was set to equal the exhaled volume.

Results

Figures 3 and 4 show the results of an exposure experiment to PER and TRI with subject 4 f at rest.

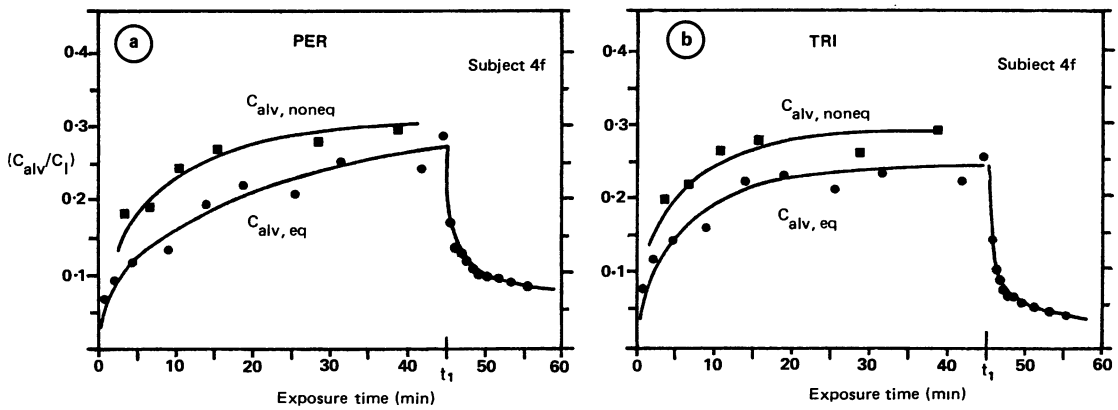

Fig 3 (a and b) *Alveolar concentration curves relative to C_I up to 60 minutes after onset of exposure for both PER and TRI for subject 4 f exposed simulateously to PER and TRI at rest. Symbols ■ and ● indicate C_{alv} relative to C_I after normal inhalation and exhalation ($C_{alv, noneq}$; method 1) and after a normal inhalation, 5 s breathholding, and normal exhalation ($C_{alv, eq}$, method 2) respectively.*

These figures are representative of the results of all subjects. Figure 3a and 3b show the C_{alv} data during the first 60 minutes after the onset of a simultaneous exposure to TRI and PER at rest.

The $C_{alv,eq}$ of PER during the limited exposure of about 45 minutes still increases, whereas that of TRI levels off. After exposure the drop of the $C_{alv,eq}$ of TRI exceeds that of PER. During exposure for both PER and TRI the $C_{alv,noneq}$ (symbol ■) exceeds the $C_{alv,eq}$ (symbol ●).

Figure 4 shows the $C_{alv,eq}$ data for TRI in the postexposure period up to 96 hours and for PER 240 hours respectively. For all the subjects the C_{alv} values of the triplos were within 4% (2–6%) and 5% (2–8%) for PER and TRI respectively.

The lines are obtained by curve fitting as described

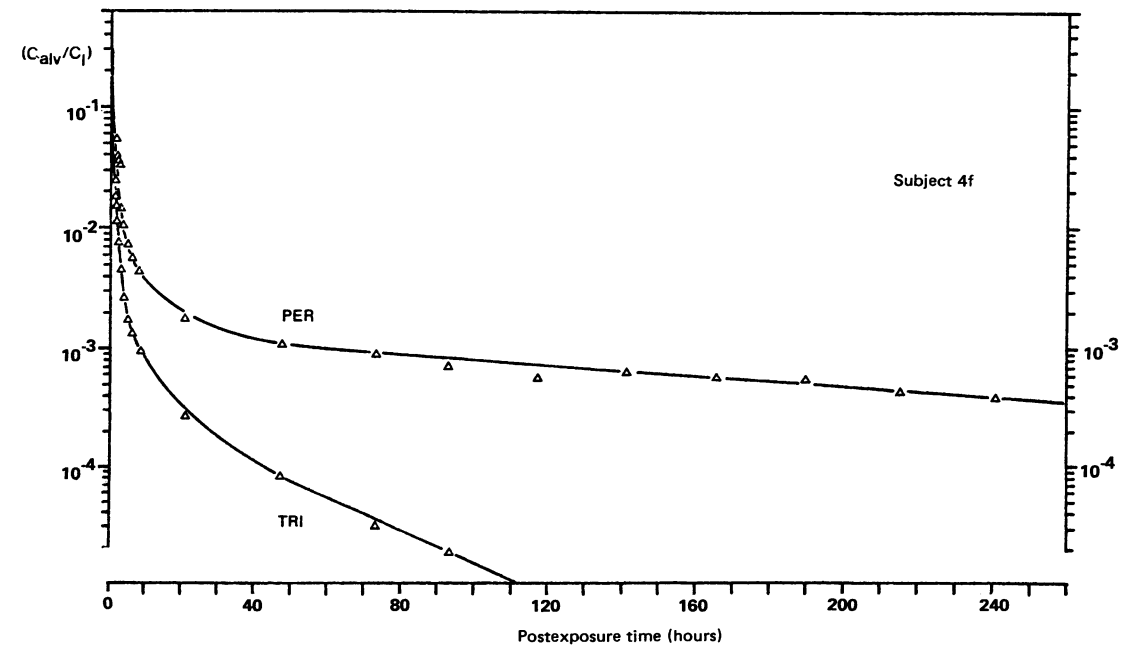

Fig 4 *Alveolar concentration curves relative to C_I in postexposure period for both PER and TRI for subject 4 f exposed simultaneously to PER and TRI at rest. Next day, samples were taken just after awakening.*

Table 3 *Coefficients c(i), exponents r(i) of subject 4 f obtained from curve fitting according to eq 2 (appendix 1) of number of n alveolar concentrations after a simultaneous exposure to PER and TRI at rest*

$c(i)/C_i$; $i = 1, \ldots, 5$		$r(i)$; $i = 1, \ldots, 5$		*No*	*n*
*PER**	*TRI*	*PER*	*TRI*	*PER*	*TRI*
0·147	0·164	−2·45	−1·55	31	27
0·079	0·057	−0·103	−0·136		
0·119	0·053	−0·0120	−0·0156		
0·087	0·020	−0·00174	−0·00218		
0·346	0·0151	−0·000086	−0·00054		

*For the poorly metabolising PER $\Sigma c(i)/C_i < 1$ because $(A1 + A2)/C_i t_i < 1$ due to $\dot{V}_A^* > \dot{V}_A$ (appendix 1).

above. The curve fitting according to equations 1 and 2 (appendix 1) resulted in several estimates of the coefficients c(i) and exponents r(i) ($i = 1, 2, \ldots p$). Table 3 shows the parameters for subject 4 f at rest.

Figure 5 shows the functional alveolar ventilation ($\dot{V}_a$) at rest, 30 W 65 W exercise for PER and TRI for all subjects calculated according to eq 6. The anatomical alveolar ventilation ($\dot{V}'_a$) is calculated with MV, f, and an assumed dead volume (V'_d) of 0·15 l (tables 1 and 2).

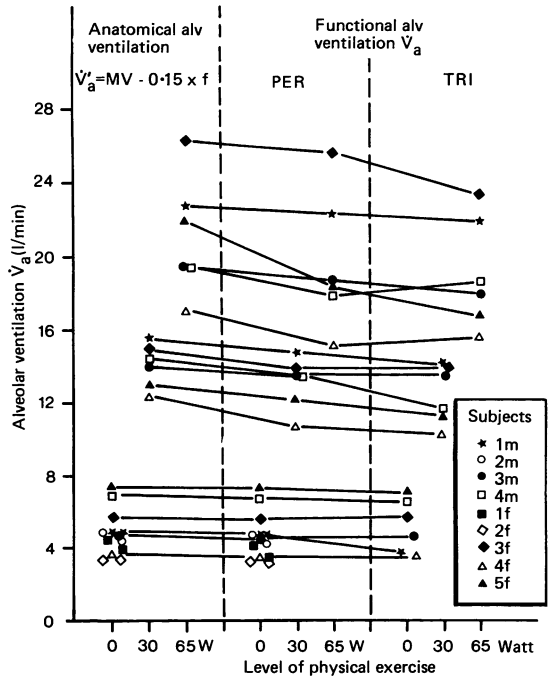

Fig 5 *Alveolar ventilation during exposure at 0, 30, and 65 Watt physical exercise for six subjects. Anatomical (V'_a) and functional ($\dot{V}_a$) alveolar ventilation are calculated by eqs 3 and 6 respectively.*

In fact, the $\dot{V}_a$ values present the intake per unit of inhaled concentration and thus at the same level of C_I the amount of solvent reaching the blood increases with physical exercise. The increase depends on the subject and ranges from a 2·5-fold up to fivefold at 65 W for the subjects 4 m and 1 m respectively. The magnitude of the interindividual range of $\dot{V}_a$ is much higher at a level of 65 W than at rest or 30 W.

Figure 5 also shows that the $\dot{V}_a$ values for TRI are somewhat smaller than for PER, particularly during exercise. This indicates a somewhat larger functional dead volume during normal respiration for TRI than for PER under conditions of exercise. At the same level of exercise the $\dot{V}_a$ values of TRI and PER differ mostly much less than 10%. The anatomical alveolar ventilation can deviate significantly from the functional alveolar ventilation within one subject depending on the level of exercise. For example, for subject 5 f the functional $\dot{V}_a$ for TRI during rest and 65 W were 4% and 24% respectively lower than the calculated anatomical $\dot{V}'_a$ with $\dot{V}'_d = 0·15$ l.

The postexposure $\dot{V}_a^*$ according to eq 10 for men after rest, 30 W, and 65 W exposure covers a range of 6·1–8·2 l/min ($\bar{x} = 7·2$, CV = 10%, n = 11); for women $\dot{V}_a^*$ covers a range of 4·1–6·8 l/min ($\bar{x} = 5·5$, CV = 17%, n = 12). As comparison the functional $\dot{V}_a$ during exposure at rest for men and women respectively covers ranges of 4·6–6·8 l/min ($\bar{x} = 5·4$, CV = 29%, n = 4) and 3·3–7·4 l/min ($\bar{x} = 4·8$, CV = 35%, n = 5) (fig 5).

Figures 6 and 7 show the exhaled fractions (F_{i1}, F_{i2}) during and after exposure relative to the functional intake for PER and TRI respectively. The exhaled fraction F_{i1} during exposure has been calculated according to eq 8a; the functional $\dot{V}_a$ is presented in fig 5. For PER with zero metabolism the postexposure fraction F_{i2} follows directly from F_{i1}; $F_{i1} + F_{i2} = 1$ (fig 6). For TRI the fraction F_{i2} is calculated according to eq 8b; $\dot{V}_a^*$ is set equal to $\dot{V}_a^*$ of PER (eq 10).

Figure 6 shows on average for the six subjects some increase of F_{i1} of PER at higher levels of exercise and therefore some decrease of F_{i2}. For the six subjects during rest the exhaled fraction F_{i1} relative to the functional PER intake covers a range of 0·11–0·27 ($\bar{x} = 0·22$) (fig 6). At 65 W exercise the F_{i1} is in the range of 0·2–0·32 ($\bar{x} = 0·26$). Therefore after rest and 65 W exercise F_{i2} covers a 0·73–0·89 and a 0·68–0·8 range respectively.

For TRI the increase of F_{i1} at a higher level of exercise is more pronounced than that of PER: at rest F_{i1} covers a range of 0·15–0·22 ($\bar{x} = 0·20$) and at 65 W a range of 0·28–0·36 ($\bar{x} = 0·33$) (fig 7). For both PER and TRI one or two subjects have a relatively large F_{i1} fraction during the 30 W exercise (figs 6 and 7).

The postexposure F_{i2} of TRI is at a low level of about 0·11–0·14. In contrast with PER after 30 and

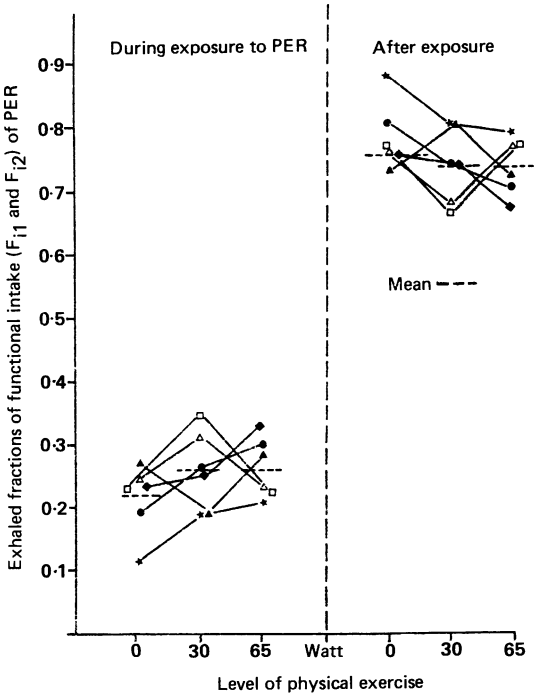

Fig 6 *Exhaled PER fractions F_{i1} and F_{i2} (eqs 8 a, b) of functional intake during and after exposure at 0, 30, and 65 W physical exercise. Each subject is presented by his own symbol (fig 5).*

65 W exercise the F_{i2} of TRI is higher (30%) than at rest: $\bar{x}$ = 0·14 and 0·11 respectively.

By definition the exhaled fraction F_u of the inert PER is set equal to 1. Figure 8 shows the F_u of TRI according to eq 11. F_u has about the same pattern as the F_{i2} in fig 7. The average F_u of TRI for the subjects equals 0·16 after rest whereas after 30 and 65 W the average F_u equals 0·21 (fig 8).

For TRI the metabolised fractions of the uptake and functional intake may be easily deduced from the total exhaled fractions in figs 7 and 8 and equal $1 - F_u$ and $1 - (F_{i1} + F_{i2})$ respectively.

Figure 9 shows that the not-exhaled fraction which is set to be equal to the metabolised fraction of the functional intake is substantially lower than that of the uptake; at rest 0·7 and 0·85, respectively. On average the metabolised fraction of the uptake after both 30 W or 65 W exercise is about 10% lower than after rest. The fractions after a 30 W and 65 W exercise are about the same. The metabolised fractions of the functional intake during and after the 30 W and the 65 W experiment are 19 and 26% lower than at the rest experiment.

Discussion

In the present study the respiratory input has been defined as a functional intake rather than as an uptake. The basic idea is that during exposure the respiratory input and excretion are considered as simultaneous processes; this has been suggested previously.[12] We define the respiratory input rate as the product of the inhaled concentration (C_I) and the functional alveolar ventilation $\dot{V}_a$ (eq 1b). $\dot{V}_a$ may be determined by using the retained amount in the body and the area under the $C_{alv,eq}$ curve during exposure (eq 6). The rate of input has now been defined in a functional way and, therefore, we call it the rate of functional intake (RFI).

Usually the respiratory input is defined as an uptake which equals the retained amount (U_{tot}) of the solvent in the body during exposure. The rate of uptake may be determined from the difference between the inhaled and exhaled amount per minute (eq 7). In that case it is implicitly assumed that the respiratory excretion only occurs after exposure.

From a kinetic point of view the metabolised fraction of the respiratory input is of interest because this fraction appears to indicate the alterations in the rate of metabolism due to, for example, physical exercise or combined exposure. The metabolised fraction equals the metabolised amount relative to the respiratory input. In general the metabolised fraction may be determined directly from amounts of the metabolites excreted in urine and indirectly from the exhaled fraction. In our experiments the second

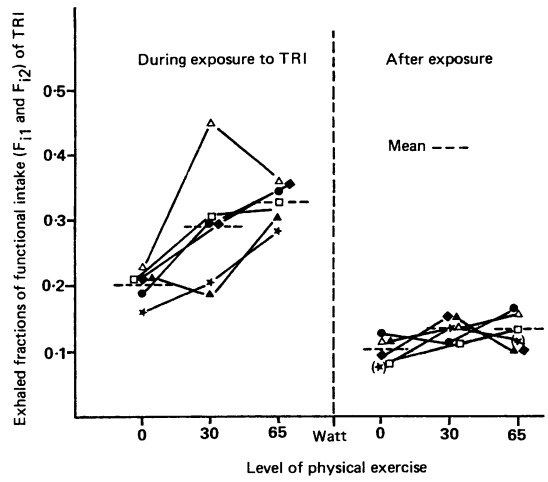

Fig 7 *Exhaled TRI fractions F_{i1} and F_{i2} (eqs 8 a, b) of functional intake during and after exposure at 0, 30, and 65 W physical exercise. Each subject is presented by his symbol (fig 5). Data between parentheses are obtained with an observation period of less than 48 hours.*

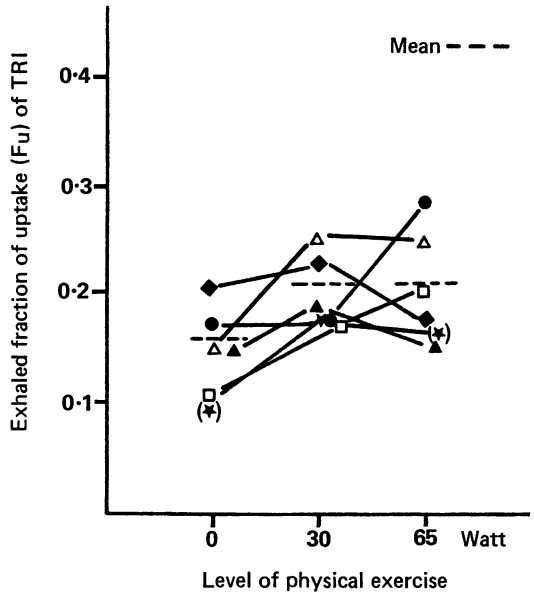

Fig 8 *Exhaled TRI fraction F_u (eq 11) of uptake after exposure at 0, 30, and 65 W physical exercise. Each subject is presented by his symbol (fig 5). Data between parentheses are obtained with an observation period of less than 48 hours.*

method was applied. The metabolised fraction relative to the uptake (F_{mu}) and the functional intake (F_{mi}) have been set equal to $1 - F_u$ and $1 - (F_{i1} + F_{i2})$ respectively.

The consequences of using respectively the rate of uptake and the RFI as respiratory input will be discussed by means of (*a*) the dependance of the metabolised fraction on the duration of exposure under conditions of rest and physical exercise and (*b*) the dependance of the kinetic response on the duration and route of administration. Furthermore, the metabolised fractions F_{mu} and F_{mi} during physical exercise as indicators of altered rate of metabolism will be discussed.

UPTAKE

The uptake as respiratory input yields some misleading conclusions. The first is that the metabolised fraction of the uptake increases with increasing duration of exposure (t_l). With a constant $C_{alv,eq}$ level the retained amount (U_{tot}) is directly proportional to t_l whereas the area under the exhaled postexposure curve (appendix 1; eq 4) is only proportional to t_l with factor $1 - \exp(r(i).t)$; (r(i) = neg). This means that with increasing t_l the exhaled amount increases more slowly than the amount U_{tot} does and therefore the relative magnitude of biotransformation increases.

An example can be given with the data of subject 4 f (table 3). Assuming a nearly constant $C_{alv,eq}$ level after two hours exposure to TRI then for an increase of the exposure duration from two to five hours the uptake increases about 2·5-fold. The postexposure exhaled amount presented by the area A2 under the curve (appendix 1; eq 4) increases 2·1-fold. Thus the fraction of the uptake metabolised increases about 15%.

Our experiments with an exposure duration in the range of 30–60 minutes showed that on the average 16% (F_u = 0·16) of the TRI uptake was exhaled after rest, whereas after 30 and 65 W exercise 21% was exhaled (fig 8). Experiments with an exposure duration of four hours both at rest and combined with 2 × 0·5 h 100 W exercise resulted on average in a 10% exhaled fraction of the uptake.[1] One may conclude that the exhaled fraction of the uptake decreases about 50% from 0·2 down to 0·1 with increasing duration of exposure from about one to four hours. Therefore the metabolised fraction may increase 12% from 0·8 up to 0·9.

It should be noted that for agents with shorter halftimes—that is larger r(i) values—the effect of the duration of exposure on the metabolised fraction will be more pronounced.

Simulation by a pharmacokinetic model also showed that the exposure duration in the range of two to five hours has a striking effect on the ratio of the fractions of uptake exhaled and metabolised.[13]

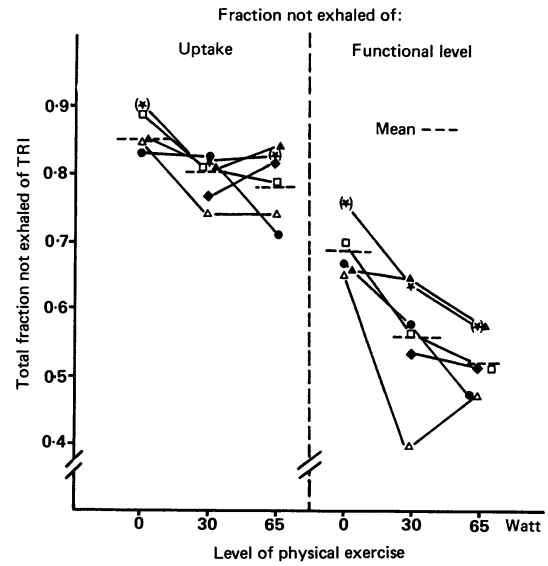

Fig 9 *Metabolised fractions of TRI calculated from the exhaled fractions. At left metabolised fraction F_{mu} of the TRI uptake: $F_{mu} = 1 - F_u$ (fig 8). At right metabolised fraction F_{mi} of the functional intake: $F_{mi} = 1 - (F_{i1} + F_{i2})$ (fig 7). Data between parentheses are obtained with an observation period of less than 48 hours.*

The dependance of F_u and F_{mu} on the duration of exposure will also hold with exposure under physical exercise conditions.

The second misleading conclusion is that the kinetic response depends on the route and the duration of administration. For example, an intravenous infusion with the same rate as the rate of uptake, the same duration and consequently the same dose as amount (U_{tot}) will give a lower kinetic response in the blood. This difference is due to the instantaneous pulmonary excretion out of the mixed venous blood during intravenous administration and can be significant for solvents with a rather low blood/air partition coefficient (λ). The excreted amount during exposure may be a substantial fraction. For example, for TRI with $\lambda = 10$ the exhaled amount during intravenous administration may be estimated as about 20–40% of the dose. In fact this is in agreement with the given fractions in figs 6 and 7. Any impact of the duration of exposure on the kinetic response appears to be clear.

INTAKE

The use of the RFI as the respiratory input rejects the above mentioned misleading conclusions. Both the total intake and the total exhaled amount—that is, represented as the sum of the areas during and after exposure (appendix 1, eq 5)—are directly proportional to the duration of exposure t_1. This means that the relative magnitude of metabolism (F_{mi}) does not depend on the duration of exposure. It should be noted that this conclusion is expected from linear kinetics. In fact, the pulmonary excretion occurs during both an intravenous infusion and a respiratory exposure and, therefore, both routes of administration are expected now to give identical kinetic responses independent of the duration. Perhaps a small difference may be expected due to the transport from the arm vein back to the pulmonary artery, but this transport is very fast and hardly any absorption occurs in the vein.

Physical exercise during exposure will change the kinetics and increases the pulmonary excretion (eq 1a). In this case the independence of both the total exhaled fraction and the metabolised fraction (F_{mi}) of the intake on the duration of exposure needs some further investigation.

It can be shown that when during exposure with exercise the steady state has been reached the exhaled fraction ($= F_{i1} + F_{i2}$) does not depend on the duration of exposure (appendix 1). In practice of experimental studies most solvents will not reach a steady state but for most solvents an almost constant $C_{alv,eq}$ level may be expected within one to four hours. Therefore before steady state the exhaled F_{i1} hardly depends on the duration t_1. Hence the postexposure fraction F_{i2} does still depend on the duration t_1 because F_{i2} depends on the total body burden at the end of exposure. The contribution of F_{i1} to the total exhaled fraction, however, may dominate that of F_{i2} as in the case of TRI (fig 7). It may be concluded that depending on the solvent only a limited duration of exposure under conditions of exercise is required in getting a near independence of the exhaled fraction or metabolised fraction on the duration of exposure.

PHYSICAL EXERCISE AND RATE OF METABOLISM

This section deals with the question whether the metabolised fraction of the respiratory input indicates alterations in the rate of metabolism due to physical exercise.

The metabolised fractions of the uptake and intake are considered separately. The behaviour of the metabolised fractions of the respiratory input are explained by means of the ratio between the rate of metabolism and the rate of respiratory input. In fact the metabolised fraction equals the ratio of the time integrals of both rates.

In general during a sufficiently long exposure the ratio between the rate of metabolism (μmol/min) and the rate of uptake (μmol/min) increases from zero to one at steady state (SS). Under SS conditions the ratio equals one and does not contain any information about changes in the rate of metabolism. Before the SS conditions have been reached it can be made clear that the changes in the rate of metabolism are hardly reflected in the metabolised fraction of the uptake (F_{mu}). During exposure the rate of uptake depends on the solvent concentration in mixed venous blood which depends on the rate of metabolism and the distribution among the organs/tissues. An increase of the rate of metabolism causes an increase in the rate of uptake. With physical exercise the $C_{alv,eq}/C_I$ increases more sharply and the SS conditions will be reached more rapidly. As a consequence at the same duration of exposure with higher levels of exercise the ratio between the rate of metabolism and the rate of uptake increases more rapidly to the constant value of one and, therefore, F_{mu} will be artificially overestimated irrespective of the rate of metabolism.

The experiments show that for TRI the exhaled fraction F_u after exposure increases 25% from about 0·16 at 0 Watt to 0·21 at 30 and 65 W (fig 8). The F_u after a 30 and a 65 W exercise remains on the same level. As a result the metabolised fraction of the uptake (F_{mu}) decreases only 7% from 0·86 (86%) down to 0·80 (80%) (fig 9).

The small impact of physical exercise during exposure on F_{mu} has been observed.[1,2] The F_{mu} covered a range of 3–6% in the first five hours after the onset of exposure with three different patterns of exercise during four hours exposure to TRI.[2]

From the above discussion it seems plausible that the fractions F_u or F_{mu} of the uptake are not able to

reflect alterations in the rate of metabolism during exposure.

During exposure the ratio between the rate of metabolism (μmol/min) and the rate of intake (μmol/min) generally increases from zero to a constant less than one when the steady state has been reached. At steady state this constant approximates the metabolised fraction of the intake because at steady state the sum of the metabolised fraction and the exhaled fraction equals one: $F_{mi} = 1 - C_{alv,eq}/C_I$ where $F_{il} \approx C_{alv,eq}/C_I$ (eq 8a). An increase of the rate of metabolism causes a decrease of $C_{alv,eq}$ and consequently an increase of F_{mi}. The $C_{alv,eq}/C_I$ ratio only depends on the rate of metabolism and the constant ($= F_{mi}$) fully reflects changes in the rate of metabolism. Furthermore, at steady state F_{mi} may be considered as a functional alveolar retention: $F_{mi} = (C_I - C_{alv,eq})/C_I$.

During exposure with physical exercise both the intake and the $C_{alv,eq}$ are expected to increase. In our experiments with TRI and PER at a higher level of exercise (0–65 W) the $C_{alv,eq}$ increased less than the intake as shown in figs 6 and 7 by means of the limited increase of the fraction F_{il} of the intake according to eq 8a. Changes in F_{il} greatly affect the metabolised fraction F_{mi} of the intake because $F_{i1} > F_{i2}$ (fig 7). At physical exercise before the SS has been reached the increase of $C_{alv,eq}/C_I$ ($\approx F_{il}$) does not indicate altered kinetic processes; one has to consider the kinetic response ($C_{alv,eq}$) relative to the respiratory input.

For the metabolising TRI the increase of F_{il} is more pronounced than for the poorly metabolising PER (figs 6 and 7); therefore the decrease of the kinetic response relative to the intake is less pronounced. At rest we found functional alveolar retentions of TRI according to eq 12 of 0·78–0·84, whereas at 65 W the retention was in the range of 0·59–0·72. A decrease of the retention at higher exercise has been found before.[1,14] Nevertheless, the less pronounced increase of $C_{alv,eq}$/RFI for TRI cannot be explained only by the rate of metabolism because the increase of the volume of distribution for TRI may be smaller than for PER. In our experiments the metabolised fraction F_{mi} decreases at higher levels of exercise (fig 9) probably mainly due to the higher pulmonary clearance during exposure.

To study the changes of the rate of metabolism, in general the use of F_{mi} is preferred above the F_{mu} for both non-steady state conditions but in particular for the steady state conditions.

For several solvents that are not highly soluble in fatty tissues, an exposure of two to four hours may be sufficient to approach the SS conditions. The rate of metabolism is reflected by ($1 - C_{alv,eq}/C_I$) which equals the functional alveolar retention or the uptake/intake ratio. When the metabolite has to be regarded as the active toxic agent, then the fraction F_{mi} permits a more adequate risk assessment than the F_{mu}.

By analogy with an intravenous administration with an input rate equal to $C_I\dot{V}_a$ a change of C_{ven} relative to the input may be due to altered pulmonary clearance, altered solvent distribution among tissues/organs, or altered metabolic clearance, or a combination of these. The amount in the body at the end of administration equals the total input minus the metabolic and pulmonary excretion.

In summary, the respiratory input rate has to be defined as the RFI rather than as the rate of uptake. The use of the uptake may lead to misleading conclusions on the kinetic characteristics of a solvent. The exhaled and metabolised fraction of the uptake depends on the duration of the exposure. This means that published experimental results are sometimes difficult to compare. Furthermore, an altered rate of metabolism or distribution among the tissues due to physical exercise hardly becomes manifest in the metabolised fraction of the uptake.

The RFI equals the product of the inhaled concentration and the functional alveolar ventilation ($\dot{V}_a$). The $\dot{V}_a$ is a virtual alveolar air volume per minute, which equilibrates completely with the mixed venous blood. During and after exposure the pulmonary excretion has been considered as a continuous process and is proportional to the concentration in mixed venous blood. The use of RFI cancels the above mentioned objections in respect of the uptake.

In linear kinetics the functional intake and the kinetics are independent variables. These two variables do determine the level and the course of the concentration in the blood respectively. Therefore the respiratory uptake may be seen as a dependent variable and may be calculated from the RFI and the kinetic response.

To estimate the respiratory input it may have become evident that in principle one has to estimate the RFI rather than the uptake. We like to emphasise the relevance of the in principle right definition of the respiratory input, in particular for experimental exposure to volatile solvents with pulmonary excretion.

Appendix 1

During and after exposure the following functions may be fitted to the observed $C_{alv,eq}$ data.

$$C_{alv,eq}(t) = \sum_{i=1}^{p} c(i)\,(1 - \exp(r(i)t)) \qquad (1)$$
$$t \leqslant t_1$$

$$C_{alv,eq}(t) = \sum_{i=1}^{p} c(i)\,(\exp(r(i)\,(t - t_1)) - \exp(r(i)t)) \qquad (2)$$
$$t \geqslant t_1$$

where t_1 = end of exposure; p = number of estimated coefficients (c(i)) and exponents (r(i): = neg). It is assumed that the kinetics and the respiratory clearance during and after exposure are the same and do not change. The areas under $C_{alv,eq}$ during and after exposure are obtained by integrating the functions (1) and (2) respectively:

$$A1 = AREA\,(C_{alv,eq}) = \sum_{i=1}^{p} c(i)/r(i) \times (1 - \exp(r(i)t_1)) + c(i)\cdot t_1 \qquad (3)$$
$$t \leqslant t_1$$

$$A2 = AREA\,(C_{alv,eq}) = \sum_{i=1}^{p} (-1)c(i)/r(i) \times (1 - \exp(r(i)t_1)) \qquad (4)$$
$$t \geqslant t_1$$

$$A1 + A2 = \sum_{i=1}^{p} c(i)t_1 \qquad (5)$$
$$t > 0$$

The area A2 equals the area (A5) between the $C_{alv,eq}$ (SS) and the $C_{alv,eq}$ with $C_{alv,eq}$ (SS) = $\Sigma c(i)$ (fig 10). For a non-metabolising solvent $C_{alv,eq}$ (SS) = C_I = $\Sigma c(i)$.

Different levels of physical exercise during and after exposure yield different kinetics and different alveolar ventilations ($\dot{V}_a$ and $\dot{V}_a$* respectively). In this case the coefficients c(i) and exponents r(i) in eqs 1 and 2 are not equal. In eqs 2 and 4 the c(i) and r(i) have to be replaced by c*(i) and r*(i). In contrast with eq 5 the total exhaled amount is not directly proportional to the duration of exposure t_1:

$$A1.\dot{V}_a + A2.\dot{V}_a^* = \dot{V}_a\Sigma c(i)t_1 - \dot{V}_a\Sigma c(i)/(-1)r(i) \times (1 - \exp(r(i)t_1)) + \dot{V}_a^*\Sigma c^*(i)/(-1)r^*(i) \times (1 - \exp(r^*(i)t_1)) \qquad (6)$$

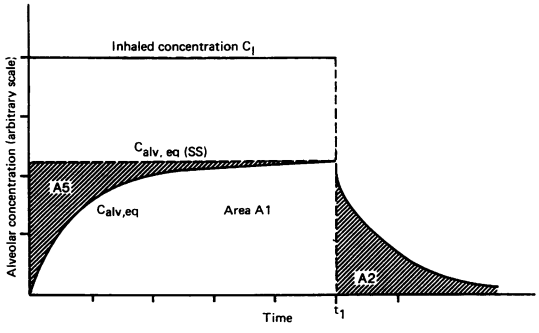

Fig 10 *Schematic times courses during and after exposure when steady state has been achieved. Areas A2 and A5 do not depend on duration of exposure t_1.*

The first term on the right side of eq 6 corresponds with the area (A_1 + A_5) under $C_{alv,eq}$ (SS), the second term with the area (A5) between $C_{alv,eq}$(SS) and $C_{alv,eq}$, whereas the third term corresponds with the post-exposure area A2. After the steady state has been reached the area A5 remains constant with prolonged exposure. The same figure holds for the area A2 because the amount in the body does not increase any more. It may be concluded that when the steady state has been reached the total exhaled amount is proportional to the first term on the right side and is directly proportional to the total intake. Therefore the exhaled fraction F_{i1} + F_{i2} does not depend on the duration t_1.

For a non-metabolising solvent total respiratory input equals total pulmonary excretion: $C_I\dot{V}_At_1$ = $A1\dot{V}_A$ + $A2\dot{V}_A$*. When $\dot{V}_A$* > $\dot{V}_A$ A2 decreases and therefore (A1 + A2)/C_It_1 < 1.

Appendix 2

Symbols and abbreviations used in the text and appendix 1.

C_{ven}	Concentration of solvent in mixed venous blood returning to the lungs (μmol/l)
$C_{alv,eq}$	Concentration in alveolar air in equilibrium with C_{ven}—that is, C_{ven}/λ (μmol/l)
$C_{alv,noneq}$	Concentration in alveolar air not in equilibrium with C_{ven} (μmol/l)
C_I	Concentration in inhaled air (μmol/l)
$\dot{V}_a$	Functional alveolar ventilation during exposure: virtual alveolar ventilation in equilibrium with C_{ven} (l/min)
V_a	Functional alveolar volume (l)
$\dot{V}_a$*	Functional alveolar ventilation in the post-exposure period (l/min)
$\dot{V}'_a$	Anatomical alveolar ventilation that is MV − f. V'_d (l/min)
V_d	Functional alveolar dead volume (l)
V'_d	Anatomical dead volume (l)
f	Respiratory frequency (l/min)
MV	Minute volume of ventilation (l/min)
RFI	Rate of functional intake (μmol/min)
RE_{pul}	Rate of pulmonary excretion (μmol/min)
$\dot{U}$	Rate of uptake (μmol/min)
U_{tot}	Retained amount in the body during exposure (μmol)
t_1	Duration of exposure (min)
λ	Blood/air partition coefficient
C_e	Average concentration in the exhaled air composed of the air in anatomical dead volume and anatomical alveolar volume (μmol/l)
A1	Area under the $C_{alv,eq}$ curve during exposure (μmol.min/l)
A2	Area under the $C_{alv,eq}$ curve after exposure (μmol.min/l)
A3	Area between the $C_{alv,noneq}$ and $C_{alv,eq}$ curves during exposure (μmol.min/l)
A4	Area between the C_I and $C_{alv,noneq}$ curves during exposure (μmol.min/l)

A5	Area between the $C_{alv,eq}$ at steady state and the $C_{alv,eq}$ curve (μmol.min/l)
F_{i1}	Exhaled fraction of the functional intake during exposure
F_{i2}	Exhaled fraction of the functional intake after exposure
F_u	Exhaled fraction of the uptake
R_{alv}	Functional alveolar retention that is $1 - F_{i1}$, at which the exhaled alveolar air is in equilibrium with C_{ven}
F_{mi}	Metabolised fraction of the functional intake that is $1 - (F_{i1} + F_{i2})$
F_{mu}	Metabolised fraction of the uptake
c(i), r(i) i = 1, 2 . . .	Estimated coefficients and exponents obtained from the curve fitting of the post exposure $C_{alv,eq}$ data.

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