Int Arch Occup Environ Health (1992) 64:383–387

International Archives of
Occupational and Environmental Health
© Springer-Verlag 1992

# Human inhalation pharmacokinetics of chlorodifluoromethane (HCFC22)

**B. H. Woollen**[1], **J. R. Marsh**[1], **J. D. Mahler**[1], **T. R. Auton**[1], **D. Makepeace**[2], **J. Cocker**[3], **and P. G. Blain**[4]

[1]Human Toxicology Team, ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TJ, UK
[2]ICI Chemicals and Polymers Ltd, Runcorn, Merseyside, UK
[3]Health and Safety Executive, Occupational Medicine and Hygiene Laboratories, 403 Edgware Road, London NW2 6LN, UK
[4]Department of Environmental and Occupational Medicine, University of Newcastle-on-Tyne, UK

Received May 27 / Accepted August 3, 1992

**Summary.** Two groups of three male volunteers were exposed to atmospheric concentrations of either 327 or 1833 mg $m^{-3}$ chlorodifluoromethane (HCFC22) for 4 h. Blood, urine and expired air samples were taken during and after the exposure period and analysed for HCFC22. Urine samples were also analysed for fluoride ion. During the exposure period, blood concentrations of HCFC22 approached a plateau, and the average peak blood concentrations of 0.25 and 1.36 μg $cm^{-3}$ were proportional to dose. HCFC22 concentrations in expired air were similar to the exposure concentration during the exposure period. The ratio between venous blood and breath concentrations of HCFC22 towards the end of the exposure period was on average 0.77, which is consistent with in vitro estimates of the partition coefficient. In the post-exposure period, three phases for the elimination of HCFC22 were identified, with estimated half-lives of 0.005, 0.2 and 2.6 h. HCFC22 was detected in urine samples taken in the post-exposure period, and the rate of decline was consistent with the terminal rate of elimination estimated from blood and breath measurements. On average 2.1% of the inhaled HCFC22 was recovered in breath within 26 h of exposure. This is consistent with the low solubility in blood and fat. Minimal changes in fluoride ion concentrations in urine following exposure indicate that HCFC22 is unlikely to be metabolised to a significant extent. Following inhalational exposure HCFC22 is poorly absorbed and is rapidly eliminated from the body. Possible biological monitoring strategies could be based on measurements of HCFC22 in urine or breath samples collected after the end of an exposure period.

**Key words:** Experimental human exposure – Pharmacokinetics – Biological monitoring – Chlorodifluoromethane

## Introduction

Chlorodifluoromethane (FC22 or HCFC22) is used commericially as a refrigerant and low temperature solvent (boiling point −41°C) and as a chemical intermediate. Human exposure may occur during manufacture or use, for example, during the servicing of refrigeration plant. In a recent review it was concluded that in over 50 years of commerical use of HCFC22 there have been few reports of adverse health effects. The lack of toxic effects reported in man and the results from extensive toxicity testing in animals demonstrate the low toxic hazard to man at HCFC22 exposure levels found in industry or in the community (ECETOC 1989).

This study was designed to establish data on the uptake and elimination of HCFC22 in human volunteers at exposure concentrations below the current Occupational Exposure Standard at 3500 mg $m^{-3}$ (Health and Safety Executive 1991). This information was used to evaluate possible biological monitoring techniques for HCFC22. A pilot study in which a single volunteer was exposed to 1380 mg $m^{-3}$ for 4 h was carried out prior to the study (data not shown).

## Materials and methods

*Protocol.* The protocol was approved by an independent ethics committee and was carried out in accordance with the 1983 Venice Amendment of the Declaration of Helsinki.

*Subjects.* The six subjects were ICI employees who had given written informed consent to participate in the study. Prior to exposure they received a full medical examination including electrocardiography, spirometry and standard haematological, biochemical and urine screening tests. These were repeated the week after exposure to HCFC22. Two groups of three subjects were exposed to target concentrations of 100 and 500 ppm (equivalent to 354 and 1770 mg $m^{-3}$) for a 4-h period.

*Exposure chamber.* The exposure chamber (2.5 × 2.5 × 2.5 m) has been described previously (Wilson et al. 1983). Test atmospheres were generated by introducing HCFC22 into the inlet duct of the exposure chamber. The concentrations in the chamber were monitored by infra-red and gas chromatographic methods. Volunteers entered the chamber at 20-min intervals to facilitate blood and breath sampling. During the exposure period they engaged in sedentary activities. No alcohol was permitted for 24 h before and until 24 h after exposure.

*Blood sampling.* During the first 12 h blood samples were collected from an indwelling intravenous cannula (Venflon) inserted into a

*Correspondence to:* B. H. Woollen

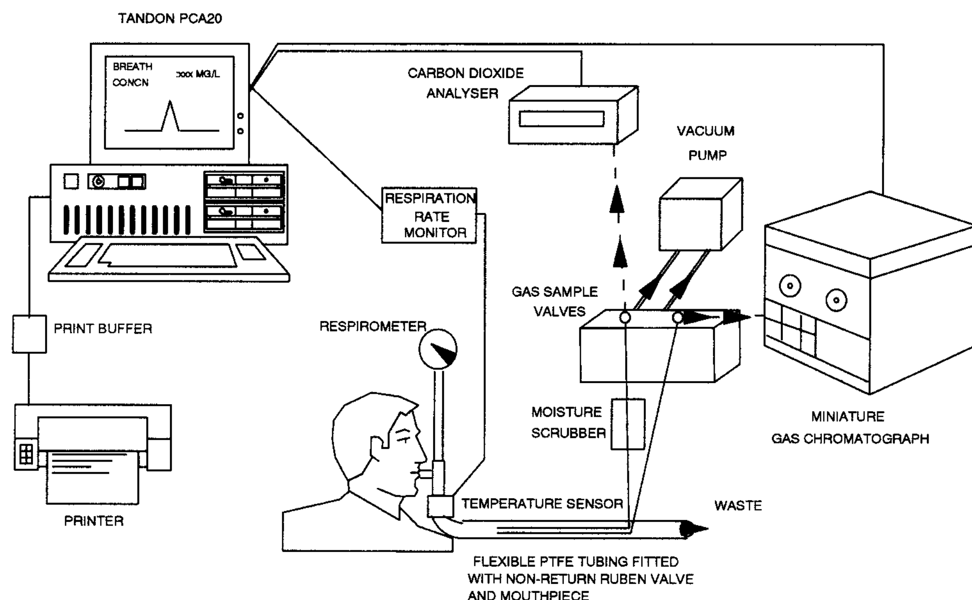


**Fig. 1.** Schematic diagram of breath analysis system

forearm vein. Samples were collected prior to exposure and after 0.5, 1, 2, 3, 3.5 and 3.97 h exposure to HCFC22. Further samples were taken immediately after the end of exposure and 0.25, 0.5, 0.75, 1, 2, 2.5, 3, 3.5, 4, 5, 5.5, 6, 7, 8, 9 and 22 h after the end of the exposure period.

*Urine sampling.* Urine samples were collected pre-exposure and for the periods 0–4.5, 4.5–6.5, 6.5–8.5, 8.5–10.5, 10.5–12.5 and 12.5–22 h. The following procedure was used to minimise evaporative losses of HCFC22. Immediately after voiding, volunteers filled a 'blood-gas' syringe with urine and fitted a sealing cap. The syringe was stored at ambient temperature until immediately prior to analysis.

*Analysis of blood and urine.* Calibration standards were prepared by serially diluting pure HCFC22 to prepare a series of standard atmospheres. Appropriate amounts of these standards were added to 2 $cm^3$ volumes of control blood or urine samples in sealed screw cap vials from which 2 $cm^3$ of air had been removed. Test and calibration vials were mixed on a roller-mixer for at least 10 min. Using a gas-tight syringe, 100 μl headspace was injected into a 1.5 × 3 mm glass column containing 0.1% SP1000 on Carbopack C (80–100 mesh), installed in a Shimadzu Mini-3 gas chromatograph, with a flame ionisation detector, nitrogen as carrier gas. Injector, oven and detector temperatures were 100°, 50° and 100°C, respectively. HCFC22 eluted with a retention time of 1.3 min. The limit of detection in blood and urine was 0.005 and 0.003 mg $l^{-1}$, respectively. Fluoride ion was determined using an ion-selective electrode.

*Breath sampling.* End-tidal breath samples were collected off-line during exposure at 0.5-h intervals. The subject fitted a nose-clip and exhaled into a snorkel-type mouthpiece connected to a Ruben valve and a heated PTFE tube (25 mm inner diameter). Breath samples were collected from a septum sampling port located 20 cm downstream from the Ruben valve. A respirometer connected to the outlet of the Ruben valve was used to establish (a) when a steady breathing pattern was achieved; (b) when to take an end-tidal breath sample from the sampling port, using a gas-tight syringe; and (c) to determine the average minute volume for each individual. Aliquots 2 $cm^3$ of the breath samples were injected into the gas chromatographic analyser.

In the post-exposure period on-line breath samples were collected using an automated system (Fig. 1). Nominal sampling times post-exposure were 1, 5, 10, 15, 20, 25, 30, 40, 50 minutes and 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6. 6.5, 7, 7.5, 8, 8.5, 9, 21 and 24 h. The subject fitted a nose-clip and exhaled into a snorkel-type mouthpiece similarly connected to a Ruben valve and a heated (40°C) PTFE tube. A thermal sensor located downstream from the Ruben valve was monitored by a computer programme to determine the respiration rate. When this had stabilised, a sample was injected into a gas chromatograph using a gas sampling valve. A sample was simultaneously injected into a carbon dioxide analyser to provide a check on the validity of end-tidal breath samples.

Breath samples collected both off-line and on-line were analysed for HCFC22 using a Shimadzu Mini-3 gas chromatograph under similar conditions to those described previously. Standard atmospheres of HCFC22, in air, were used to calibrate the analyser.

*Partition coefficients.* Human blood/air and fat/air partition coefficients of HCFC22 were determined by a headspace chromatographic method (Sato and Nakajima 1979) at concentrations of 1 and 20 μg $cm^3$ at 37°C. Donated human fat was homogenised, centrifuged, and the supernatant used undiluted for the partition coefficient measurements.

*Calculations.* The area under the blood concentration versus time curve (AUC), extrapolated to infinity, was calculated using an iterative computer programme (ELSMOS, kindly donated by R. J. Francis), which was also used to estimate the hybrid rate constants from blood and breath data. The amount of HCFC22 excreted in breath in the post-exposure period was calculated by integrating the area under the curve of a plot of excretion rate (mg $min^{-1}$) versus time, extrapolated to infinity. The elimination rate was estimated from the product of the end-tidal breath concentration (mg $l^{-1}$) and the estimated minute volume for each individual, corrected for the respiratory deadspace (0.33). The inhaled dose for each individual was calculated from the mean atmospheric concentration during the exposure period, respiratory rate and tidal volume measurements. Blood/breath ratios were obtained by dividing venous blood concentrations by the end-tidal breath concentration, both expressed in micrograms per litre.

*Physiological pharmacokinetic modelling.* A physiologically based pharmacokinetic model (Auton and Woollen 1991) was used to gain a further insight into the dispositon of HCFC22. The model incorporates partition coefficients from in vitro experiments and predicts disposition between different body compartments.

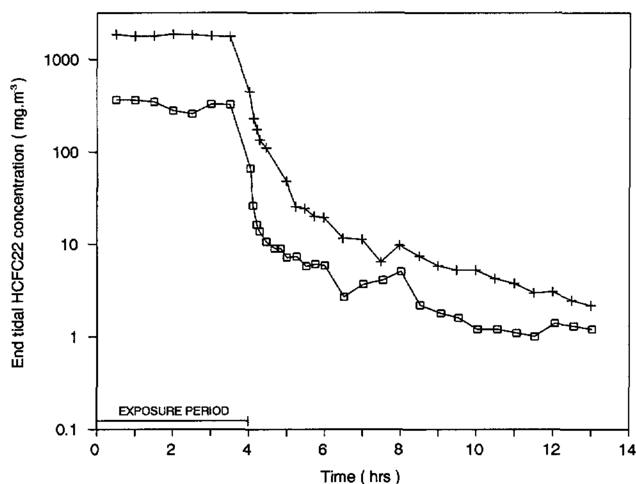


**Fig. 2.** Mean end-tidal breath concentrations of HCFC22 following exposure to the nominal concentrations shown ($n = 3$). + Mean exposure concentration 1833 mg $m^{-3}$; □ mean exposure concentration 327 mg $m^{-3}$

## Results

The atmospheric concentrations of HCFC22 (± SD) were 327 ± 3 and 1833 ± 2 mg $m^{-3}$, which correspond to 92 and 518 ppm. There were no adverse effects on the volunteers during or after exposure to HCFC22.

Blood concentrations of HCFC22 approached a plateau within 1 h of exposure, and mean (± SD) peak concentrations of 0.25 ± 0.015 and 1.36 ± 0.03 μg $cm^{-3}$ were proportional to dose. In the post-exposure period there was a rapid intitial decline, followed by a slower decline in blood concentrations.

Breath concentrations measured during exposure were very similar to the exposure concentrations from 0.5 h onwards. In the post-exposure period breath concentrations declined steadily in most instances (Fig. 2). An apparent rise in breath concentration after 6–8 h for the 327 mg $m^{-3}$ group is believed to be due to a small degree of re-exposure due to the ventilation characteristics of the building where the study was carried out.

HCFC22 was detectable in urine and the highest concentrations were found in the 0–4.5 h samples (0.05–0.07 and 0.28–0.42 μg $cm^{-3}$ for the 327 and 1833 mg $m^{-3}$ exposure groups, respectively). Thereafter urinary concentrations of HCFC22 declined rapidly to non-detectable levels in the low-dose group, but HCFC22 was still detectable in samples collected between 12.5 and 22 h for the 1833 mg $m^{-3}$ group (range 0.003–0.016 μg $cm^{-3}$). Urinary fluoride concentrations were all below the normal upper limit for control subjects of 2 mg $l^{-1}$ (Massmann 1981).

The breath elimination curves for HCFC22 during the first 9 h following exposure can be described by a three-compartment pharmacokinetic model. Following the exposure period there was a very rapid decline in breath concentrations (mean $t_{1/2}$ 0.005 h), followed by two slower phases with average half-lifes of 0.17 and 2.4 h (Table 1). The average amount of HCFC22 recovered in breath in the post-exposure period was 2.0% (range 2.0%–2.7%) of the estimated amount inhaled. Since minimal amounts were found in urine this is consistent with very low systemic absorption of HCFC22.

Blood results are described by a two-compartment open model, and estimated elimination rates are shown in Table 1. The rate of elimination of HCFC22 in urine was estimated for the 1837 mg $m^{-3}$ group only, for the period 4.5–12.5 h (Fig. 3), and the estimated half-lives are shown in Table 1. Estimates of α, β and γ elimination rates of HCFC22 from blood, breath and urine data are within good agreement (Table 1).

**Table 1.** Summary of pharmacokinetic parameters in subjects 2–7

| | 2 | 3 | 4 | Mean 2–4 | 5 | 6 | 7 | Mean 5–7 | Mean 2–7 |
|---|---|---|---|---|---|---|---|---|---|
| Dose (mg $m^{-3}$) | 330 | 327 | 324 | 327 | 1833 | 1833 | 1830 | 1833 | NA |
| Breath | | | | | | | | | |
| α Half-life (h) | 0.005 | 0.001 | 0.001 | 0.002 | 0.005 | 0.006 | 0.01 | 0.007 | 0.005 |
| β Half-life (h) | 0.16 | 0.08 | 0.05 | 0.10 | 0.25 | 0.25 | 0.23 | 0.24 | 0.17 |
| γ Half-life (h) | 3.0 | 2.6 | 1.7 | 2.4 | 3.0 | 2.3 | 2.2 | 2.5 | 2.40 |
| Blood | | | | | | | | | |
| α Half-life (h) | 0.29 | 0.18 | 0.26 | 0.24 | 0.27 | 0.09 | 0.26 | 0.21 | 0.23 |
| β Half-life (h) | 2.8 | 1.9 | 2.1 | 2.3 | 4.6 | 2.4 | 2.9 | 3.3 | 2.8 |
| AUC (μg h $ml^{-1}$) | 0.329 | 0.231 | 0.275 | 0.278 | 1.18 | 1.04 | 1.65 | 1.29 | NA |
| Urine | | | | | | | | | |
| α Half-life (h) | ND | ND | ND | NA | 3.8 | 1.8 | 2.2 | 2.6 | NA |
| Breath, Blood and Urine[a] | | | | | | | | | |
| α (h) | | | | | | | | | 0.005 |
| β (h) | | | | | | | | | 0.20 |
| γ (h) | | | | | | | | | 2.6 |

ND, Not determined; NA, not applicable

[a] β-phase for breath corresponds to α-phase for blood; γ-phase for breath corresponds to β-phase for blood and α-phase for urine

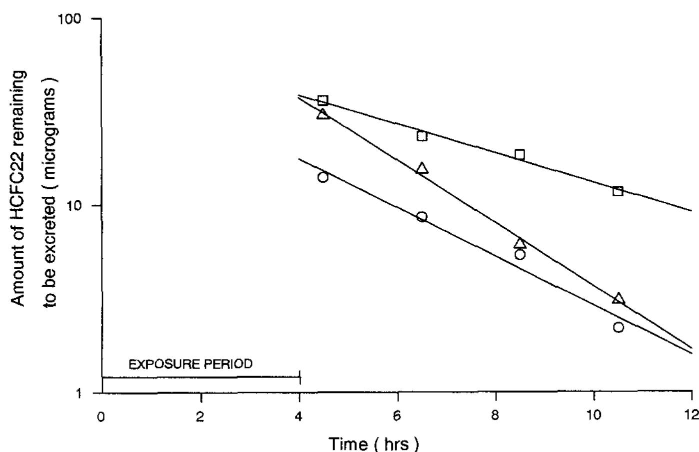


**Fig. 3.** Semi-logarithmic plot of urinary excretion of HCFC22 following exposure to 1833 mg m$^{-3}$. Lines shown are calculated by linear regression. □ Subject 5; △ subject 6; ○ subject 7

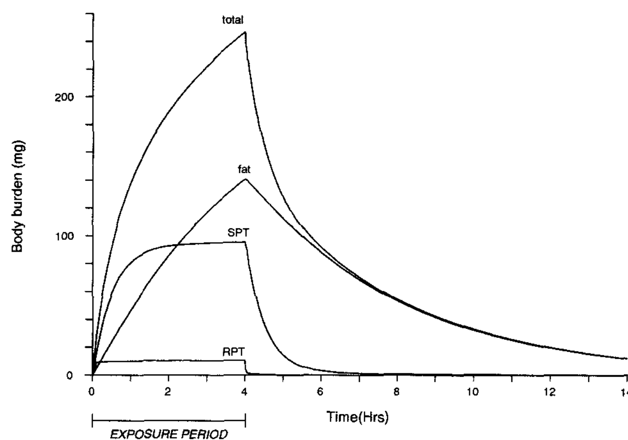


**Fig. 4.** Model prediction of the distribution of body burden of HCFC22 into different compartments during and following a 4-h exposure to 1770 mg m$^{-3}$ RPT: rapidly perfused tissues, blood and viscera. SPT: slowly perfused tissues, muscle and skin

The mean blood/air partition coefficients for HCFC22 at concentrations of 1 and 20 µg ml$^{-1}$ were determined to be 0.82 and 0.76. These are in good agreement with literature values of 0.80 (Varene et al. 1989) and 0.67 (Franks et al. 1989). The fat/air partition coefficients determined at the same concentrations were 7.7 and 8.1, respectively. From these results the fat/blood partition coefficient was estimated to be 10.

## Discussion

During a 4-h exposure to HCFC22 blood concentrations rapidly approach a plateau. This is consistent with the low solubility of HCFC22 in blood, as evidence by the average blood/gas partition coefficient of 0.79. The mean ± SD venous blood/breath ratio calculated during the second half of exposure period of 0.78 ± 0.09 is in excellent agreement with the in vitro measurement. Average peak blood concentrations were directly proportional to exposure concentration. The ratio of blood AUCs was not significantly different ($P > 0.05$, Student's $t$ test) from the ratio of the exposure concentration (5.6).

In the immediate post-exposure period, breath concentrations of HCFC22 declined very rapidly, on average by a factor of 5 within the first 2 min. Blood concentrations declined more slowly, by less than a factor of 2 within the first 5 min. The likely explanation for these observations is that initially HCFC22 is rapidly cleared from the airways, blood and well perfused tissues, causing a rapid fall in the breath concentrations. The second phase of elimination involves removing HCFC22 from the slowly perfused tissues. Venous blood samples taken from the arm are no longer in equilibrium with arterial blood, and are likely to reflect the removal of HCFC22 from the slowly perfused tissues. After the first few minutes breath concentrations also fall more slowly, reflecting removal from the slowly perfused tissues.

A disposition diagram derived using the mathematical model (Fig. 4) predicts a pattern which is consistent with the experimental data and provides a useful tool in understanding the pharmacokinetics of HCFC22. The model simulation predicts that at the end of the exposure period only a small fraction of HCFC22 is distributed in the central compartment, which corresponds to blood and rapidly perfused tissues. A greater proportion is present in slowly perfused tissues, and the highest proportion is present in fat. At the end of the exposure period the model predicts that HCFC22 would be cleared very rapidly from the central compartment, corresponding to the initial rapid fall in breath concentrations. Subsequently the amount present in slowly perfused tissues falls quite rapidly, and a minimal amount remains in this compartment 2 h after the end of the exposure period. This corresponds to the second phase seen for the breath measurements and the first phase determined for blood. The model indicates that the final phase observed for measurements in blood breath and urine, corresponds to release from fat, and that more than 90% of the HCFC22 absorbed is eliminated within 10 h of the end of exposure. The relatively rapid elimination from fat is a consequence of the relatively low fat/blood partition coefficient, which was estimated to be 10. This contrasts markedly with FC113, a compound, which we have studied previously (Woollen et al. 1990) which has a similar solubility in blood to HCFC22 but a much higher solubility in fat.

The average amounts of HCFC22 recovered in breath in the post-exposure period were 18.7 and 95.1 mg for the two dose levels. In all cases this is only a very small proportion (< 2.7%) of the amount inhaled during the exposure period. Another possible route of elimination of HCFC22 is urine. Since HCFC22 is soluble in water to the extent of 0.3% at 25°C, it is not surprising that detectable amounts were found in urine. The elimination half-life for excretion of HCFC22 in urine (mean value 2.8 h) was similar to that determined for the second phase of elimination from blood. The amount of HCFC22 excreted in urine was extremely small (average values 0.02 and 0.15 mg for the two exposure groups), demonstrating that although HCFC22 can be detected,

urine is not quantitatively significant as a route of excretion compared to exhalation. However, the concentrations of HCFC22 measured in urine 30 min after the end of the exposure showed a good correlation with the administered dose (average values of 0.347 and 0.065 mg $l^{-1}$ (ratio 5.3 : 1) at analysed exposure concentrations of 1833 and 327 mg $m^{-3}$ (ratio 5.6 : 1).

The absence of significant change in urinary fluoride excretion following exposure indicates that HCFC22 is not metabolised to a significant extent. This is consistent with the observations following inhalation exposure to HCFC22 in the rat (Peter et al. 1986).

The results of this study suggest two possible methods for the non-invasive assessment of HCFC22 absorption. Breath analysis could be used with a sample being taken at least 30 min after the end of the exposure period. Alternatively, measurements of HCFC22 in urine samples collected at the end of the exposure period could be investigated. Further work would be needed to validate either of these approaches.

The results of this study demonstrate low uptake of HCFC22 during a 4-h inhalation exposure at concentrations below the current Occupational Exposure Standard, and concentrations of HCFC22 in blood approach a steady-state concentration which is proportional to the exposure concentration. Following termination of exposure, elimination of HCFC22 is rapid. Based on the amount of HCFC22 recovered in breath, only 2% of the amount inhaled was absorbed during the exposure period. Accumulation is not likely to occur with repeated exposure.

*Acknowledgements.* The authors gratefully acknowledge the assistance of Dr. H. K. Wilson (atmosphere generation and analysis), Miss H. Shearer (blood and urine analysis), Mr. N. J. Loftus (pharmacokinetic analysis), Mr. R. Kilvington and Mr. D. Addison (breath analysis system). The study was sponsored by the European Center for Toxicology (ECETOC) and ICI Chemicals and Polymers Ltd.

## References

Auton TR, Woollen BH (1991) A physiologically based pharmacokinetic model for the human inhalation pharmacokinetics of 1,1,2-trichloro-1,2,2,-trifluoroethane. Int Arch Occup Environ Health 63 : 133–138

ECETOC (1989) Joint assessment of commodity chemicals no. 9 chlorodifluoromethane. ECETOC, Brussels

Franks PJ, Hooper RM, Jones PRM, (1989) Solubility of Freon-22 in blood and lung tissue. Br J Anaesth 62 : 425–428

Health and Safety Executive (1991) Occupational exposure limits. Guidance note EH40/91. HMSO, London

Massmann W (1981) Reference values for the renal excretion of fluoride. J Clin Chem Clin Biochem 19 : 1039–1041

Peter H, Filser JG, Szentpaly L, Wiegand HJ (1986) Different pharmacokinetics of dichlorofluoromethane (CFC21) and chlorodifluoromethane (CFC22). Arch Toxicol 58 : 282–283

Sato A, Nakajima T (1979) Partition coefficients of some aromatic hydrocarbons and ketones in water, blood and oil. Br J Ind Med 36 : 231–234

Varene N, Choukroun ML, Martman R, Verene P (1989) Solubility of Freon-22 in human blood and lung tissue. J Appl Physiol 66(5) : 2468–2471

Wilson HK, Robertson SM, Waldron HA, Gompertz D (1983) Effect of alcohol on the kinetics of mandelic acid excretion on volunteers exposed to styrene vapour. Br J Ind Med 40 : 75–88

Woollen BH, Guest EA, Howe W, Marsh JR, Wilson HK, Auton TR, Blain PG (1990) Human inhalation pharmacokinetics of 1,1,2-trichloro-1,2,2-trifluoroethane. Int Arch Occup Environ Health 62 : 73–78