Arch Toxicol (1992) 66: 1 – 10

Archives of
Toxicology

Review

The closed chamber technique – uptake, endogenous production, excretion, steady-state kinetics and rates of metabolism of gases and vapors

J. G. Filser

GSF – Forschungszentrum für Umwelt und Gesundheit, Institut für Toxikologie, Ingolstädter Landstraße 1, D-8042 Neuherberg (München), FRG

Received 25 March 1991/Accepted 31 July 1991

Abstract. The "closed chamber technique" (CCT) is presented. It allows investigation of pharmacokinetics of volatile substances in vivo in animals and in man and in vitro using tissue fractions. During the exposure period only the atmospheric concentrations of the substance are measured. The concentration-time data obtained are pharmacokinetically analyzed by a two compartment model describing uptake, endogenous production and excretion of the unchanged substance and its metabolic elimination. Using this model, pharmacokinetics of ethylene have been determined in rats and man. For both species, the results compared well with an estimation based on an allometric species scaling. Furthermore, the applicability of CCT is demonstrated in vivo on several other gases and vapors of solvents, e.g. trichloroethylene and 1,1,1-trichloroethane, and in vitro on 1,2-epoxybutene-3.

Key words: Closed chamber technique – In vivo system – In vitro system – Inhalation – Pharmacokinetics – Species scaling – Ethylene – Trichloroethylene – 1,1,1-Trichloroethane – 1,2-Epoxybutene-3

Introduction

The potency of toxic or carcinogenic agents depends on their tissue dose. It usually relies on the rates of uptake by, distribution in, and elimination from the exposed organism. Furthermore, many xenobiotics are not effective by themselves but by reactive metabolites. Mostly, biotransformation to and metabolic elimination of such metabolites are subject to saturation kinetics. Such processes are described by pharmacokinetics. Thus, knowledge of the pharmacokinetics of a substance is indispensable for understanding and extrapolating dose-response relationships and for species scaling of the effective dose. In vivo and in vitro studies are necessary for evaluation of the pharmacokinetic parameters.

Pharmacokinetic in vivo studies of gases and vapors have to consider both the processes of distribution and elimination as well as the uptake via inhalation and via skin. Exposure systems used for such "gas uptake" studies are "open" or "dynamic" and "closed" systems. In the former, a continuous gas flow maintains the exposure concentration constant and the gas concentrations are measured in the inspired and in the expired air, e.g. for studies with humans: Fiserova-Bergerova and Teisinger (1965); Stewart et al. (1968); Nomiyama and Nomiyama (1974); Fernandez et al. (1976); Monster et al. (1976); Buchter et al. (1978); Wigaeus et al. (1981); Åstrand (1982); Nolan et al. (1984); Johanson (1986); Liira et al. (1990); for studies with animals: Peter and Bolt (1984); Freundt et al. (1989). In the closed system in contrast, a certain amount of the gas or solvent is usually injected only once at the start of the experiment, e.g. in Hefner et al. (1975); Bolt and Filser (1977); Filser and Bolt (1979); Andersen et al. (1979); Kessler et al. (1989); Gargas et al. (1990). Thereafter, gas concentrations in the atmosphere of the chamber are monitored repeatedly. They change during the exposure period due to uptake, metabolic elimination and, occasionally, endogenous production.

For analyzing the concentration-time courses determined in the atmosphere of the closed chambers a physiologically based pharmacokinetic model (Gargas et al. 1986) or a compartment model (Filser and Bolt 1979, 1983, Kessler et al. 1989) is used. The former is especially appropriate for species extrapolation if the tissue/blood partition coefficients for the different tissues and the kinetic parameters of the metabolizing enzymes are known. The compartment model gives fewer insights into the behavior within the organism, but yields parameters describing the measured curves and provides information on rates of inhalation, exhalation, metabolic elimination and endogenous production much more precisely.

In this communication, the closed chamber technique (CCT) and its application in animals and man is demonstrated on different gases and vapors. The information available by analyzing the measured data by a two-compartment models is elaborated. Furthermore, for studying

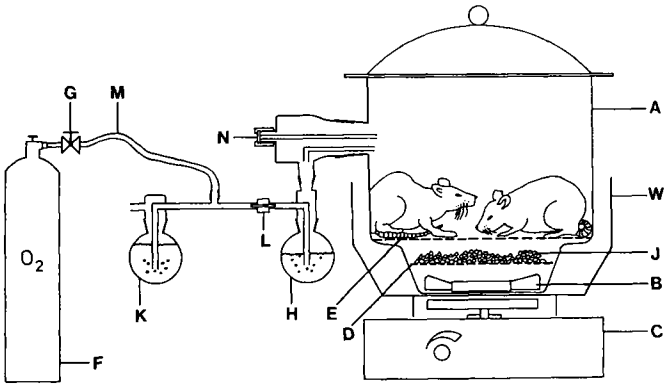

Fig. 1. Closed all-glass system for exposing laboratory animals. Symbols are specified in text

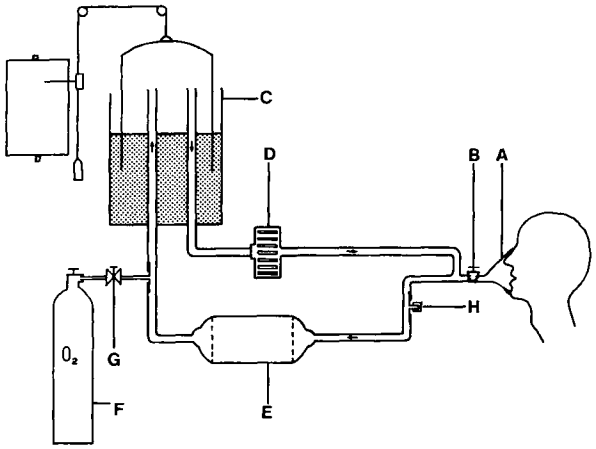

Fig. 2. Spirometer system for exposing humans to hydrophobic volatile substances. Symbols are specified in text

enzyme dependent kinetics of volatile substances in vitro, CCT together with the two compartment model is very convenient. Using 1,2-epoxybutene-3 as substrate, this is demonstrated on microsomes and cytosol from livers of mouse, rat, and man.

Materials and methods

Closed exposure systems

Three types of closed chambers are presented: the rodent system, the human system, and the in vitro apparatus.

Rodent system. This system to expose rats and mice (Fig. 1) (Filser and Bolt 1981) is a modification of that described by Filser and Bolt (1979) and Link (1979) which is based on the system of Bolt et al. (1976): In a glass desiccator (A) of volume between 0.85 l and 24 l acccording to the experimental requirement, the animals were placed on a punched sheet of stainless steel (E). Desiccators with relatively small air space compared to animal volume are preferred for measuring the exhalation of endogenous substances, since the concentration-time curve reaches concentration of plateau faster than in larger chambers (e. g. Filser et al. 1983). In contrast, use of wide desiccators is more convenient if uptake of highly accumulating gases as 2-nitropropane vapor has to be determined, since concentration decline in the gas phase is relatively slower, allowing measurements of higher precision (Kessler et al. 1989). In most cases, the desiccator contained 135 g soda lime (J) on a stainless steel wire lattice (D) to absorb the exhaled CO_2. A teflon coated iron fan (B) driven by a magnetic stirrer (C) enabled circulation of the air in the chamber. The chamber was immersed in a temperature controlled water bath (W) to maintain a constant temperature of 25° C in the chamber.

Since the loss of the exhaled CO_2 was automatically compensated by oxygen, no underpressure occurred in the chamber. The oxygen was delivered subsequently by a special device as shown in Fig. 1: oxygen (F) flowed into a system consisting of two water traps (K, H). Only the amount necessary to replace the oxygen consumed flowed into the chamber; excess oxygen escaped into the laboratory.

The test substance was injected via a teflon coated rubber septum (N) into the atmosphere of the chamber or administered to the animals intraperitoneally or orally, immediately before starting the experiment. To determine the concentration-time course in the atmosphere of the chamber, aliquots of its air were taken for gas chromatographic analysis.

A modified system allows collection of urine and feces separately (Hallier et al. 1981).

Human system. This system is based on a spirometer system developed to determine the pharmacokinetics of vinyl chloride in man (Buchter et al. 1978). The "Spirotest Junior" spirometer (Jaeger, Würzburg, FRG) was modified by replacing the original soda lime container by an all-glass device of 5 l volume (Fig. 2). In addition, rubber parts were replaced by teflon and glass tubing. By a breathing mask (A) 'Rhinomaske' (Jaeger, Würzburg) connected to a valve (B), volunteers were linked to the gas room of the system (12 l, C) for exposure periods of up to 4 h. Exhaled air was mixed with the chamber atmosphere by an electric pump (70 l/min; D) and was cleaned free of CO_2 in the container (E) by 1 kg soda lime. The trapped CO_2 was replaced by the same volume of pure oxygen (F) via a valve (G). Concentrations of the gases in the system were measured by taking air samples through a teflon-coated septum (H) for gas chromatography.

This system permits determination of the pharmacokinetic parameters of non-water soluble gases and vapors in man with a minimal body burden: if the initial concentration of a xenobiotic in the 12 l chamber atmosphere is 100 ppm, this amount is equivalent to that of a test material inhaled during a 8 h exposure to 0.5 ppm assuming an alveolar ventilation of 5 l/min.

In vitro apparatus. In 1976 Kappus et al. presented an apparatus to measure uptake, metabolism, and endogenous production of volatile substances in cells and cell fractions (Kappus et al. 1976; Bolt and Filser 1977; Bolt et al. 1977a, 1978, 1979; Siegers et al. 1978; Kappus and Muliawan 1982; Stacey and Kappus 1982; Muliawan et al. 1982; Ottenwälder et al. 1983). Based on this system we developed the in vitro apparatus as shown in Fig. 3 (Kreuzer et al. 1991). It comprises the storage desiccator (A), the distributor (B) with eight incubation vessels of 42 ml each (C) containing the incubation medium and the cells or cell fractions. A suitable amount of the test substance was volatized in the desiccator (A). The incubation vessels were cooled in an ice-bath and B and C were evacuated with a water jet pump. Then the valves between A and B were opened to achieve the same gas concentration in the whole system. Finally, the valve on A was opened for a moment, so the entire system came to atmospheric pressure. After closing their valves, the incubation vessels were disconnected and incubated in a 37° C shaking water bath. According to Sato and Nakajima (1979a), the concentration-time courses of the test material in the vessel headspace were monitored by taking small air samples through teflon-covered rubber septa and analyzing them with a gas chromatograph.

Gas chromatography

In most cases gas chromatographs equipped with gas sampling loops of 0.5–10 ml were used. Separation was done isothermally on stainless steel columns of 1/4 or 1/8 inch packed with Porapack Q, Tenax GC or with specially treated alumina as described in Filser et al. (1983). Peaks

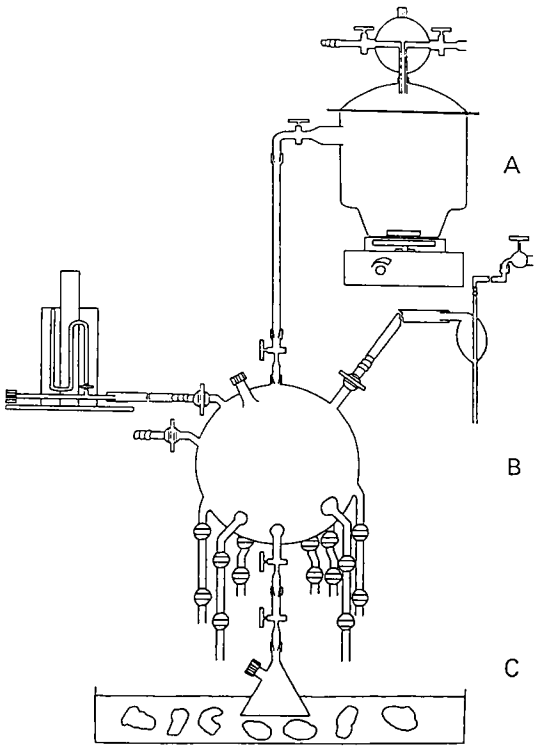

Fig. 3. In vitro apparatus for gas uptake studies with cells and cell fractions. Symbols are specified in text

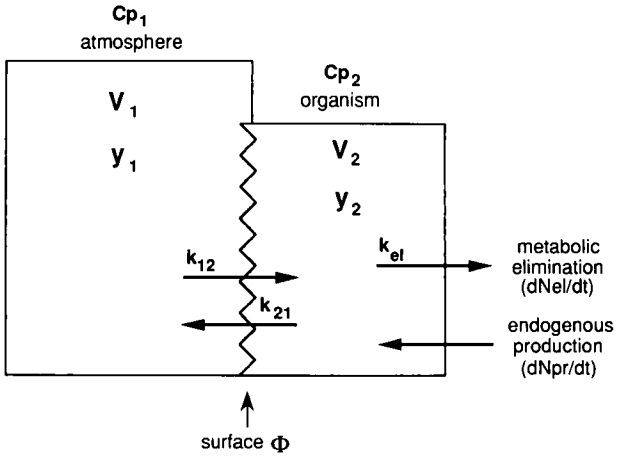

Fig. 4. Pharmacokinetic two compartment model for the closed exposure system.
Cp_1 compartment 1 representing the atmosphere of the closed chamber
Cp_2 compartment 2 representing the exposed organism, and cells or cell fractions, respectively
V_1 volume of Cp_1
V_2 volume of Cp_2
y_1 concentration in Cp_1
y_2 concentration in Cp_2
k_{12} microconstant of the uptake process into Cp_2
k_{21} microconstant of the exhalation or evaporation process from Cp_2
k_{el}* concentration dependent microconstant of the metabolic elimination process within Cp_2
Φ surface separating the two compartments

were identified with flame ionization detection by their characteristic retention time.

Pharmacokinetic analysis

The atmospheric concentration-time data measured were analyzed by means of the two compartment pharmacokinetic model presented in Fig. 4. The equations related to the model have been published in a series of papers (Filser and Bolt 1979, 1981, 1983; Filser et al. 1983; Bolt et al. 1981, 1984; Bolt and Filser 1987; Kessler et al. 1989).

The atmosphere of the closed chamber is considered to be the first compartment (Cp_1). It has volume V_1 and atmospheric concentration y_1. The second compartment (Cp_2) with volume V_2 and average concentration y_2 represents the whole organism exposed, and cells or cell fractions in incubations in vitro, respectively. The exchange rates between both compartments are directly proportional to y_1 and y_2, respectively. The proportionality factors are k_{12} and k_{21}. The factor linking y_2 to the actual rate of metabolism is called k_{el}*. In contrast to k_{12} and k_{21}, both of which have constant values, the value of k_{el}* can change with the concentration y_2. It is constant only if linear pharmacokinetics occur. However, if metabolism underlies saturation kinetics, k_{el}* becomes smaller with increasing y_2 (see below). Furthermore, an endogenous production of the test substance is included into the pharmacokinetic model. The amount of substance produced endogenously per time step (dt) is given by the expression dN_{pr}/dt. The definitions of the symbols used are summarized in the legend to Fig. 4.

The following differential equations are related to the model:

$$V_1 \cdot dy_1/dt = -k_{12} \cdot V_1 \cdot y_1 + k_{21} \cdot V_2 \cdot y_2 \quad (1)$$

$$V_2 \cdot dy_2/dt = k_{12} \cdot V_1 \cdot y_1 - (k_{el}^* + k_{21}) \cdot V_2 \cdot y_2 + dN_{pr}/dt \quad (2)$$

They describe the change of the amount of test substance in the atmosphere and in the organism, respectively.

The compartments are separated by a surface Φ. The rates of uptake into the organism from the gas phase (dN_{up}/dt) and of exhalative elimination of the unchanged substance into the atmosphere (dN_{ex}/dt) are assumed to be proportional to the area of the separating surface, to the actual concentration in the atmosphere and to the actual average concentration in the organism, respectively:

$$dN_{up}/dt = \alpha \cdot \Phi \cdot y_1 \quad (3)$$

$$dN_{ex}/dt = \beta \cdot \Phi \cdot y_2 \quad (4)$$

The proportionality factors α and β are permeation constants according to Fick's law. They depend on temperature, gas pressure, biophysical properties of the surface Φ and on physicochemical properties of the test substance such as its solubility. Furthermore, both processes can be expressed with respect to the volumes of the compartments and the corresponding microconstants:

$$dN_{up}/dt = k_{12} \cdot V_1 \cdot y_1 \quad (5)$$

$$dN_{ex}/dt = -k_{21} \cdot V_2 \cdot y_2 \quad (6)$$

The proportionality factors $k_{12} \cdot V_1$ and $k_{21} \cdot V_2$ are called "clearance of uptake", and "clearance of exhalation", respectively. Both parameters are linked by the thermodynamic equilibrium constant K_{eq}:

$$K_{eq} = k_{12} \cdot V_1 / (k_{21} \cdot V_2) \quad (7)$$

K_{eq} is the concentration ratio whole organism/atmosphere which can directly be measured if no endogenous production and no metabolism of the test material occur ($dN_{pr}/dt = 0$, $k_{el}^* = 0$):

$$K_{eq} = (y_{1(0)} - y_{1(\infty)}) \cdot V_1 / (y_{1(\infty)} \cdot V_2) \quad (8)$$

The symbols $y_{1(0)}$ and $y_{1(\infty)}$ represent the initial and the final ($t \to \infty$) concentrations of the test material in the atmosphere of the closed chamber.

Both parameters, k_{12} and k_{21} are dependent on the exposure conditions:

Since clearance of uptake from the atmosphere $k_{12} \cdot V_1$ equals the constant α (eqns 3 and 5), it becomes obvious that $k_{12} \cdot V_1$ is independent of the volume V_1 of the exposure chamber: $k_{12} \cdot V_1$ is identical in closed and in open chambers where y_1 remains constant. In other words, at a

given gradient over the surface ϕ, the amount of substance passing through ϕ per time is constant and not dependent on the atmospheric volume V_1. Consequently, k_{12} is in inverse proportion to V_1. Furthermore, k_{12} and k_{21}, are dependent on the surface Φ. Thus, if for instance two animals are exposed instead of one, both V_2 and the surface Φ are doubled. Here, the surface Φ is proportional to the number n of the animals. On the other hand, if equal numbers n of animals with different volumes V_2 are used, their surfaces are assumed to be proportional to the individual animal volume (V_2/n) to the 2/3 power. Therefore, Φ is proportional to $n\cdot(V_2/n)^{2/3}$. Along with eqns 4 and 6, the clearance of exhalation $k_{21}\cdot V_2$ can be expressed to be:

$$k_{21}\cdot V_2 = \gamma\cdot n\cdot(V_2/n)^{2/3} \quad (9)$$

In a second experiment with different exposure conditions (k'_{21}, V'_2, n', k'_{12}, V'_1), one obtains:

$$k'_{21}\cdot V'_2 = \gamma\cdot n'\cdot(V'_2/n')^{2/3} \quad (10)$$

An expression for k'_{21} can be obtained based on k_{21}, V_2, and n, by combining eqns 9 and 10 and eliminating γ:

$$k'_{21} = k_{21}\cdot[V_2\cdot n'/(V'_2\cdot n)]^{1/3} \quad (11)$$

For optional exposure conditions, this equation allows calculation of the corresponding clearance of exhalation $k'_{21}\cdot V'_2$.

The rate constant k'_{12} is in inverse proportion to V'_1 but depends on Φ (see above). Considering eqn 7, it becomes obvious that

$$k'_{12}\cdot V'_1/(k'_{21}\cdot V'_2) = k_{12}\cdot V_1/(k_{21}\cdot V_2) \quad (12)$$

Inserting eqn 7 in eqn 12 and solving it to $k'_{12}\cdot V'_1$ one obtains:

$$k'_{12}\cdot V'_1 = K_{eq}\cdot k'_{21}\cdot V'_2 \quad (13)$$

Both eqn 11 and eqn 13 have been applied to standardize clearances of uptake and of exhalation determined in experiments under different conditions. Metabolic elimination from the compartment organism (dN_{el}/dt) is given by

$$dN_{el}/dt = -k_{el}^*\cdot V_2\cdot y_2 \quad (14)$$

It can follow different types of kinetics. This is reflected by k_{el}^*, the introduction of which allows to describe diverse metabolic processes. It is not treated as concentration independent constant but only as a factor the value of which depends on the exposure conditions. For instance, eqns 15–17 show three expressions which had been used for k_{el}^* up to now (Kessler et al. 1989):

$$k_{el}^* = V_{max}/[V_2\cdot(Km_{app} + y_2)] \quad (15)$$
$$k_{el}^* = V_{max}/[V_2\cdot(Km_{app} + y_2)] + k_{el} \quad (16)$$
$$k_{el}^* = V_{max1}/[V_2\cdot(Km_{app1} + y_2)] + V_{max2}/[V_2\cdot(Km_{app2} + y_2)] \quad (17)$$

Equations 15 and 17 describe one and two saturable processes, respectively, according to Michaelis-Menten kinetics. The maximum rates of metabolism are expressed by V_{max}, V_{max1}, and V_{max2}. The apparent Michaelis-Menten-constants related to the average concentrations in the organism y_2 are given by Km_{app}, Km_{app1}, and Km_{app2}. Equation 16 describes two processes, a saturable one according to Michaelis-Menten kinetics and a non-saturable one within the concentration range investigated according to first order kinetics with the rate constant k_{el}. This second linear process could also express a non-enzymatic reaction.

At low concentrations, the rate of metabolism is directly proportional to the average concentration in the organism (y_2). The proportionality factor $k_{el}^*\cdot V_2$ is called "clearance of metabolism". Its value is directly proportional to V_2. This has to be taken into account in comparing experiments carried out under different conditions and for standardization.

Both $k_{21}\cdot V_2$ and $k_{el}^*\cdot V_2$ can be used to calculate for steady state conditions the rates of elimination of the unchanged substance, and of metabolism in an open system ($V_1 \to \infty$) with constant concentration y_1. For this, they have to be related to y_1 by multiplying with K_{st}^*.

K_{st}^* is the bioaccumulation factor which gives the concentration ratio organism/air at steady state conditions. It is concentration dependent if saturation kinetics occur. It increases with growing concentration and approaches K_{eq} at high concentrations where metabolism is saturated. K_{st}^* times y_1 gives the average concentration in the organism y_2 at steady state (open system, y_1 = const.). K_{st}^* is expressed by:

$$K_{st}^* = (k_{12}\cdot V_1\cdot y_1 + dN_{pr}/dt)/[(k_{el}^* + k_{21})\cdot V_2\cdot y_1] \quad (18)$$

Using $k'_{12}\cdot V'_1$, k'_{21}, dN'_{pr}/dt, and V'_2 the standardized K'^*_{st} is obtained. According to eqn 18 the standardized rates of exhalative elimination dN'_{ex}/dt and of metabolism dN'_{el}/dt can be related to the atmospheric concentration at steady state:

$$dN'_{ex}/dt = -k_{21}\cdot V'_2\cdot K'^*_{st}\cdot y_1 \quad (19)$$
$$dN'_{el}/dt = -k_{el}^*\cdot V'_2\cdot K'^*_{st}\cdot y_1 \quad (20)$$

Normally, the endogenous production dN_{pr}/dt is considered to be constant during the exposure period and to be directly related to the volume of the animals. Therefore, it is standardized to:

$$dN'_{pr}/dt = dN_{pr}/dt\cdot V'_2/V_2 \quad (21)$$

Using this compartment model, the program 'SOLVEKIN' (Csanády and Filser 1990) calculates concentration-time curves which match the measured values. The program is written in C and runs under VMS, Unix, MSDOS, Mac OS, and MIPS OS. It solves the differential equations of the model numerically by one of several implemented methods which can be chosen by the user. Then, the program computes the pharmacokinetic parameters using the method of the least error squares and the simplex algorithm of Nelder and Mead (1965) for function minimization. The stepsize is controlled automatically. To obtain the confidence intervals of the estimated parameters, the curvature of the sum-squared error function is examined near the minimum. Although the model comprises two compartments only, excellent fits are usually obtained. The estimated pharmacokinetic parameters can be introduced into a physiologically based pharmacokinetic model reducing the number of variables needed in such models (Johanson and Filser, paper in preparation).

Results

Concentration-time courses in closed exposure systems

The exposure conditions in closed systems differ from those in open systems. Decreasing atmospheric concentrations in a closed system result in higher accumulations of the gas at each actual atmospheric concentration than constant gas concentrations in an open system. Because of this effect, the metabolic elimination falls below V_{max} – which only depends on the concentration in the body – at lower atmospheric concentrations than in an open system.

Figures 5 and 6 show concentration-time courses characteristic for gas uptake studies in the closed chamber. Figure 5 is a semilogarithmic plot of two concentration-time courses measured in the atmosphere of closed exposure chambers of 10.3 l in each of which three Wistar rats were exposed to 1,1,2-trichloroethene. The test substance was injected as a liquid into the atmosphere of the chambers to yield – after vaporization – concentrations of 1900 ppm (points) and of 1500 ppm (squares). The lower curve (points) can be divided into three phases. The first phase is characterized by a rapid decline of the atmospheric concentration. This is mainly due to the accumulation of the solvent in the organism, since at high concentrations the metabolizing enzyme system is saturated (V_{max} conditions). That the first phase represents the accumulation in the organism is proven by the upper curve which was obtained with animals pretreated i.p. 1 h prior to the experiment with 150 mg/kg 6-chloro-1,2,3-benzothiadiazole as

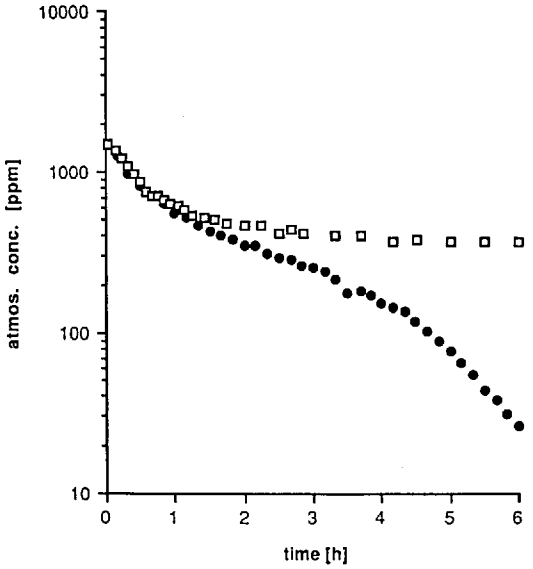

Fig. 5. Concentration-time courses of 1,1,2-trichloroethene in the atmosphere of closed exposure chambers of 10.3 l in each of which three male Wistar rats were exposed simultaneously. *Points:* untreated animals; *squares:* animals pretreated with 6-chloro-1,2,3-benzothiadiazole (150 mg/kg)

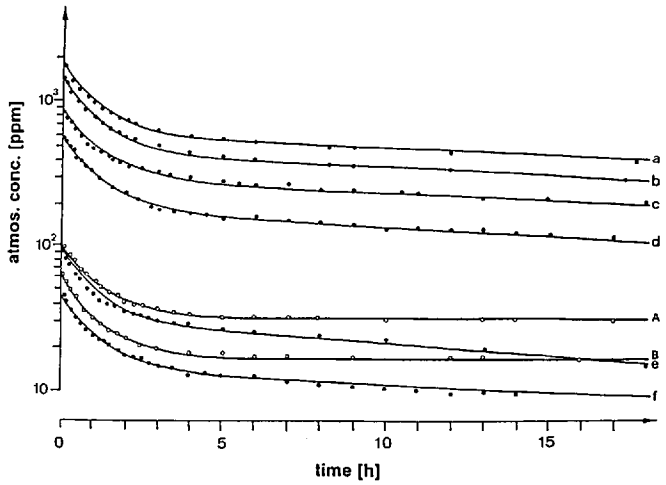

Fig. 6. Concentration-time courses of 1,1,1-trichloroethane in the atmosphere of closed exposure chambers of 6.4 l in each of which two male Wistar rats were exposed simultaneously. *a–f:* untreated animals. *A, B:* animals pretreated with dithiocarb (200 mg/kg)

described in Bolt et al. (1976) to inhibit cytochrome P-450-dependent metabolism (according to Siegers et al. 1978): due to the inhibitory effect, the accumulation of 1,1,2-trichloroethene almost reached the highest possible value given by the thermodynamic equilibrium constant organism/gas phase. After this enrichment phase practically no further decline of the curve occurred. 1,1,2-Trichloroethene accumulates especially in the fat tissue, since it is highly soluble in the fatty compartment but only weakly in water (Sato and Nakajima 1979b). The initial slopes of both curves do not depend on the metabolism but only on the rate of absorption of the gas by the organism. The downward curvature in the second phase of the lower

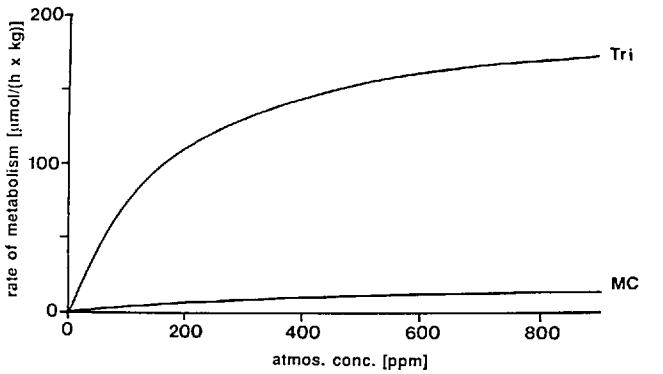

Fig. 7. Rates of metabolism of 1,1,2-trichloroethene (*Tri*) and of 1,1,1-trichloroethane (*MC*) in male Wistar rats at steady state in dependence on their atmospheric concentration

curve is typical for saturation kinetics. The third phase in this curve, below 100 ppm, is identified by a constant slope. It is almost identical with that at the start of the enrichment phase. Hereof it can directly be concluded that at low concentrations the rate of metabolism of 1,1,2-trichloroethene is limited by the rate of absorption by the organism and not by the affinity of the metabolizing enzymes. Therefore, 1,1,2-trichloroethene accumulates barely at low concentrations in contrast to high concentrations at which its metabolism is limited by the enzymic capacity (Filser and Bolt 1981).

In Fig. 6, a semilogarithmic plot of eight concentration-time courses is shown obtained with 1,1,1-trichloroethane vapors in closed chambers (6.4 l). In each experiment two rats were exposed in a desiccator to different initial concentrations of 1,1,1-trichloroethane (Filser and Deml 1989). The shape of the curves (a–f) is characteristic for saturation kinetics, since their final slopes are increasing with decreasing concentrations. The values of these final slopes are smaller than those of the initial slopes of the same curves. This directly proves that even at low concentrations the rate of metabolism of 1,1,1-trichloroethane is not limited by the inhalative uptake but by the substrate affinity of the metabolizing enzyme system. The biotransformation rates are very slow compared to 1,1,2-trichloroethene. This slow metabolism results in an enrichment of 1,1,1-trichloroethane which is almost as large as the thermodynamic equilibrium between organism and gas phase and is independent of the exposure concentration. Inhibition of the metabolism of 1,1,1-trichloroethane by administration of dithiocarb [200 mg/kg, 10 min prior to the experiment, modified according to Siegers et al. (1978)] results in a constant atmospheric concentration following the enrichment phase which leads to the thermodynamic equilibrium organism/gas phase (curves A, B).

Using the two compartment model, the rates of metabolism of 1,1,2-trichloroethene and of 1,1,1-trichloroethane at steady state in dependence on their atmospheric concentration were calculated (Fig. 7). In rats, V_{max} of 1,1,1-trichloroethane was 33, and V_{max} of 1,1,2-trichloroethene was 210 µmol/h/kg body weight. For this substance, other authors using different methods estimated V_{max} to be 132

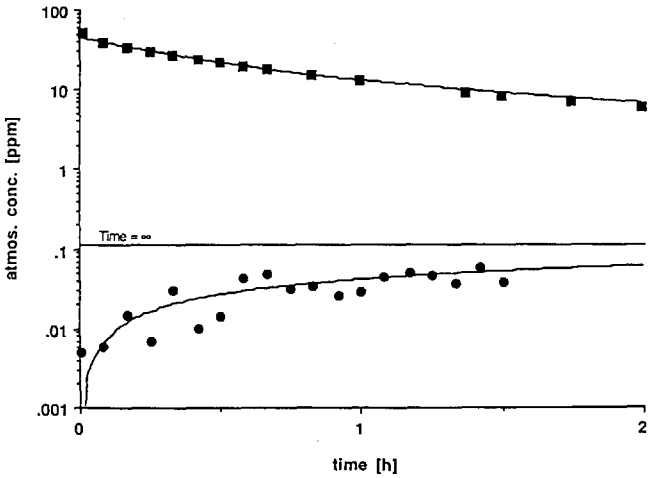

Fig. 8. Concentration-time courses of ethylene in two experiments in the atmosphere (12 l) of a spirometer system to which a male volunteer (64 kg) was linked by a breathing mask. *Squares:* measured values; initial concentration 50 ppm ethylene. *Points:* measured values; initial concentration 0 ppm ethylene. *Lines:* calculated curves

(Fisher et al. 1989), 160 [calculated from Prout et al. (1985)], and 185 (Andersen et al. 1980) µmol/h/kg body weight. The differences could derive from the use of different rat strains in these studies.

Rodent system

Since 1977 the desiccator system has been used to study the kinetics of a number of gases and vapors in laboratory animals (Bolt and Filser 1977, 1987; Bolt et al. 1977b, 1981, 1984; Buchter et al. 1978, 1980; Filser and Bolt 1979, 1981, 1983, 1984; Bolt and Link 1980; Frank et al. 1980; Hallier et al. 1981; Kivits et al. 1981; Frank and Dürk 1983; Peter and Bolt 1984; Remmer and El Majid Gharaibeh 1984; Robertson et al. 1985; Simon et al. 1985; Kreiling et al. 1986, 1987; Peter et al. 1986, 1987; Dogra et al. 1988; Golka et al. 1989; Kessler et al. 1989; Shen et al. 1989; Schwegler et al. 1990). Similar systems were used by Ehrenberg et al. (1974), Igwe et al. (1986), and by Daugherty et al. (1988). Pharmacokinetic analyses of gas uptake studies by Andersen and coworkers who also used a closed system are reviewed in Gargas et al. (1990).

The desiccator system was suitable for determining the kinetics of water soluble substances like acetone, 2-butanone and 2-nitropropane and of lipophilic halogenated hydrocarbons, alkenes, and alkanes. The system was also sensitive enough for studying the kinetics of slowly metabolized substances as ethylene or 1,1,1-trichloroethane (see above). The endogenous production of ethane and n-pentane (Filser et al. 1983), ethylene (Shen et al. 1989), isoprene (Peter et al. 1987) and acetone (Filser and Bolt 1983) was quantified by measuring their exhalation and determining the corresponding pharmacokinetic parameters. Furthermore, the exhalation of endogenously produced gaseous reactive intermediates as 1,3-butadiene monoxide and ethylene oxide has been directly shown when animals were exposed to the corresponding metabolic precursors 1,3-butadiene and ethylene, and the pharmacokinetics of the epoxides could be determined (Filser and Bolt 1984; Bolt and Filser 1987; Kreiling et al. 1987).

Human system

This system has been applied to study the pharmacokinetics of vinyl chloride (Buchter et al. 1978; Bolt et al. 1981), n-hexane (Filser et al. 1987; Kessler et al. 1990), ethylene (Shen et al. 1989; Denk 1990; Filser et al. 1991), and isoprene (Hartmann et al. 1990). The endogenous production of the two latter substances was demonstrated by exposing volunteers towards synthetic air only and measuring the enrichment of the exhaled gases in the system. Two typical concentration-time courses of ethylene are exemplified in Fig. 8. The upper curve gives the atmospheric concentrations obtained by exposing a volunteer to ethylene at an initial concentration of 50 ppm. The lower curve shows the exhalation of endogenous ethylene by the same volunteer. From the data of such inhalation and exhalation experiments the pharmacokinetic parameters and the endogenous production were calculated as described in Dogra et al. (1988). Using these parameters the straight lines in Fig. 8 were derived. The one which parallels the time axis gives the plateau concentration at which rates of production and metabolism are equal.

The alveolar retention of ethylene was estimated to be about 2% of the alveolar ventilation at steady state. The endogenous production rate was 0.5 nmol/(h·kg) leading to a body burden of 0.01 nl gas/ml tissue (Denk 1990; Filser et al. 1991)

The pharmacokinetics of ethylene in rats obtained by means of the CCT (Bolt et al. 1984; Bolt and Filser 1987; Shen et al. 1989) were compared with those obtained in man (Shen et al. 1989; Denk 1990; Filser et al. 1991). Striking species differences were observed with respect to the clearances and the rates of production of endogenous ethylene. An extrapolation from rat to man based on body weights leads to values diverging more than one order of magnitude from the actual values. However, using an allometrical method [reviewed in Davidson et al. (1986)] a much better extrapolation of the pharmacokinetic data from rat to man has been obtained: the pharmacokinetic parameters for an adult human were predicted from those obtained with rats by means of a factor of 40. This factor is almost equivalent to the difference in body surfaces when expressed as $[\text{body volume}]^{2/3}$. With the exception of the endogenous production rate, the pharmacokinetic parameters estimated differed only by a factor less than two from those observed in man. Consequently, the pharmacokinetic properties, e.g. pulmonary retention, the ratios of ethylene metabolized and of ethylene exhaled to the amounts of ethylene taken up, and the body burden with endogenous ethylene, were similar in both species (Table 1).

We obtained corresponding results comparing the species specific pharmacokinetics of n-hexane (Filser et al. 1987; Kessler et al. 1989), of 2-butanone (Kessler et al. 1989), and of ethylene oxide (Denk 1990) in rat and man and of isoprene (Hartmann et al. 1990) and styrene (Schwegler et al. 1990) in mouse, rat, and man. Obviously,

Table 1. Pharmacokinetic parameters of ethylene in one rat [$n' = 1$, $V'_2 = 250$ ml] and in one human [$n' = 1$, $V'_2 = 70 \cdot 10^3$ ml]

Parameter		Value			Dimension
Name	Expression	Rat	Man (predicted from rat[a])	Man (observed[b])	
Thermodynamic equilibrium constant (body/air)	K_{eq}[c]	0.70	0.70	0.53	$\frac{\text{nl gas/ml tissue}}{\text{ppm in atmosphere}}$
Bioaccumulation factor (body/air)	K'_{st}*	0.54[d]	0.54[d]	0.33	$\frac{\text{nl gas/ml tissue}}{\text{ppm in atmosphere}}$
Clearance of uptake (related to atmosph. conc.)	$k'_{12} \cdot V'_1$[c]	1200	$48 \cdot 10^3$	$25 \cdot 10^3$	ml/h
Clearance of exhalation (related to atmosph. conc.)	$k'_{21} \cdot V'_2 \cdot K'_{st}$*	910[d]	$36 \cdot 10^3$[d]	$16 \cdot 10^3$	ml/h
Clearance of metabolism (related to atmosph. conc.)	$k_{el}^* \cdot V'_2 \cdot K'_{st}$*	280[d]	$11 \cdot 10^3$[d]	$9.3 \cdot 10^3$	ml/h
Endogenous production rate	dN'_{pr}/dt	2.8	112	32	nmol/h
Half-life	$\ln 2/(k_{el}^* + k'_{21})$	0.079[d]	0.55[d,e]	0.65	h
$\frac{\text{Amount metabolized} \cdot 100}{\text{Amount taken up}}$	$k_{el}^* \cdot 100/(k_{el}^* + k'_{21})$	24[d]	24[d]	36	%
$\frac{\text{Amount exhaled} \cdot 100}{\text{Amount taken up}}$	$k'_{21} \cdot 100/(k_{el}^* + k'_{21})$	76[d]	76[d]	64	%
Alveolar retention	$\frac{k_{el}^* \cdot V'_2 \cdot K'_{st}^* \cdot 100}{\text{Pulm ventilation}}$	3.5[d,f]	2.5[d,g]	2.1[f,g]	%
Maximum rate of metabolism	V_{max}	2.1	84	not determined	µmol/h
Apparent Michaelis-Menten constant	Km_{app}	130	130	not determined	nl gas/ml tissue
Body burden with ethylene due to endogenous product.	y_{2ETend}[h]	0.032	0.032	0.011	nl gas/ml tissue

[a] An allometrically derived surface factor of 40 was used to extrapolate values of clearances, half-life, endogenous production rate and maximum rate of metabolism

[b] Parameters were determined at atmospheric concentrations below 50 ppm

[c] Values are independent of the atmospheric concentration

[d] Values are valid for atmospheric concentrations up to 80 ppm

[e] Half-life for man $t_{1/2(man)}$ was calculated with the equation: $t_{1/2} = \ln 2 \cdot V'_{2(man)}/[(k'_{21(rat)} + k_{el}^*{}_{(rat)}) \cdot V'_{2(rat)} \cdot 40]$

[f] Pulmonary ventilation was set to be $8 \cdot 10^3$ ml/h (according to Guyton 1947)

[g] Pulmonary ventilation was set to be $450 \cdot 10^3$ ml/h (own measurements)

[h] Body burden with endogenous produced ethylene y_{2ETend} was calculated with the equation: $y_{2ETend} = dN'_{pr}/dt \cdot 25{,}130/[1000 \cdot (k'_{21} + k_{el}^*) \cdot V'_2]$

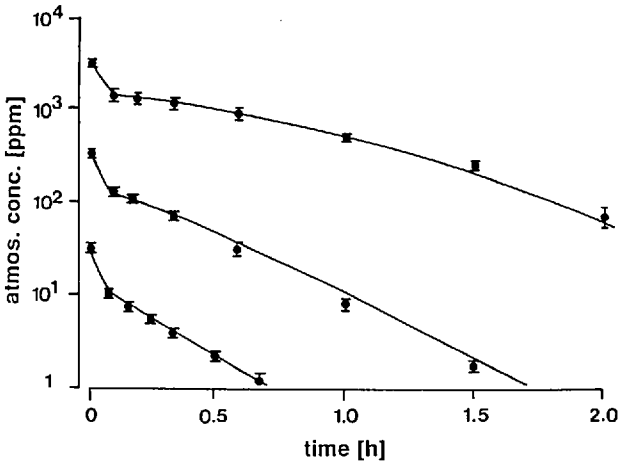

Fig. 9. Concentration-time courses at 37° C of 1,2-epoxybutene-3 vapor in the gas phase (41 ml) of closed incubation vessels containing incubates (1 ml) of human liver microsomes (3 mg protein/ml incubate). Conditions: K-phosphate buffer 150 mmol/l (pH 7.4); $MgCl_2$ 0.5 mmol/l; glutathione 15 mmol/l; NADP 0.1 mmol/l; D,L-sodium-isocitrate 0.8 mmol/l; isocitrate dehydrogenase 50 µg/ml

species scaling based on the differences in body surfaces is justifiable at least at concentrations when transport to the metabolizing enzymes is the rate limiting step.

In vitro apparatus

This system has been used to investigate kinetics of the metabolism of 1,2-epoxybutene-3 in liver fractions of mouse, rat, and man (Kreuzer at al. 1991). In Fig. 9, concentration-time courses of 1,2-epoxybutene-3 vapor are shown, which were measured in the atmosphere of the exposure vessels when liver microsomes of man were incubated. By means of the two compartment model concentrations of 1,2-epoxybutene-3 in the liquid phase, and the pharmacokinetic parameters describing saturable and non-saturable metabolism were calculated. The curves fitted by the two compartment model demonstrate the three phases characteristic for CCT: the enrichment, the saturation, and the linear phase (see above).

Epoxide hydrolase activity was found in microsomes and glutathione S-transferase activity in cytosol only. The

data obtained were used to estimate the total rate of metabolism for each species in vivo: this was calculated to be 1.3 times higher in mice and 2.3 times lower in man compared to rats, when corrected for body weight (for details see Kreuzer et al. 1991). Comparing the data scaled for mice and rats to experimental results, excellent fittings were obtained (Johanson and Filser, paper in preparation).

Discussion

From the given examples it becomes obvious that CCT offers several advantages compared to measurements in open systems.

Rodent system

Using this system the maximum accumulation in the body of laboratory animals can be determined easily: due to the decline of the atmospheric concentration the enrichment phase is terminated much earlier than in open systems in which the gas concentration remains constant during the whole exposure period. In the closed chamber wide concentration ranges can be registered in a short exposure time and saturation kinetics can be studied in animals within one exposure session. Therefore, compared with open exposure systems CCT reduces the number of laboratory animals necessary to determine the pharmacokinetics of inhaled gases and vapors. Furthermore, since the gas room of the closed chamber is relatively small compared to the volume of the exposed organisms, even marginal metabolism leads to changes in the gas concentration, allowing evaluation of the pharmacokinetic parameters of substances which are barely biotransformed.

Human system

This system provides an easy way for determining rates of inhalative uptake, of exhalative and metabolic elimination and of endogenous production of hydrophobic gases in man from inhalation and exhalation studies only. By means of CCT the pharmacokinetic parameters can be obtained with a much smaller body burden than in open systems, so that even exposures of humans are justifiable, offering the chance of verifying the validity of species-scaling procedures. In the case of ethylene and ethylene oxide the pharmacokinetic parameters obtained served to estimate the background hydroxyethylation of hemoglobin in man due to the production of endogenous ethylene (Filser et al. 1991) and for risk estimation (Denk 1990; Denk and Filser 1990), which was based on results of long term studies carried out with ethylene oxide in rats (Snellings et al. 1984; Garman et al. 1985).

In vitro apparatus

The presented apparatus was developed to adapt CCT in vitro (Kreuzer et al. 1991). By this method the parent volatile substance and not metabolites are measured and the time concentration courses are kinetically analyzed. The investigation of kinetics using CCT is easy and less time consuming than other conventional techniques in which metabolites have to be determined.

References

Andersen ME, Gargas ML, Jones RA, Jenkins LJ Jr (1979) The use of inhalation techniques to assess the kinetic constants of 1,1-dichloroethylene metabolism. Toxicol Appl Pharmacol 47: 395–409

Andersen ME, Gargas ML, Jones RA, Jenkins LJ Jr (1980) Determination of the kinetic constants for metabolism of inhaled toxicants in vivo using gas uptake measurements. Toxicol Appl Pharmacol 54: 100–116

Åstrand I (1982) Work load and uptake of solvents in tissues of man. In: Englund A, Ringen K, Mehlman MA (eds) Occupational health hazards of solvents, Vol. II. Princeton Scientific Publishers, INC, Princeton, NJ, pp 141–152

Bolt HM, Filser JG (1977) Irreversible binding of chlorinated ethylenes to macromolecules. Environ Health Perspect 21: 107–112

Bolt HM, Filser JG (1987) Kinetics and disposition in toxicology. Example: carcinogenic risk estimate for ethylene. Arch Toxicol 60: 73–76

Bolt HM, Link B (1980) Zur Toxikologie von Perchlorethylen. Verh Dtsch Ges Arbeitsmedizin 20: 463–470

Bolt HM, Kappus H, Buchter A, Bolt W (1976) Disposition of (1,2-^{14}C) vinyl chloride in the rat. Arch Toxicol 35: 153–162

Bolt HM, Buchter A, Wolowski L, Gil DL, Bolt W (1977a) Incubation of ^{14}C-trichloroethylene vapor with rat liver microsomes: uptake of radioactivity and covalent protein binding of metabolites. Int Arch Occup Environ Health 39: 103–111

Bolt HM, Laib RJ, Kappus H, Buchter A (1977b) Pharmacokinetics of vinyl chloride in the rat. Toxicology 7: 179–188

Bolt HM, Filser JG, Hinderer RK (1978) Rat liver microsomal uptake and irreversible protein binding of [1,2-^{14}C] vinyl bromide. Toxicol Appl Pharmacol 44: 481–489

Bolt HM, Filser JG, Wiegand M, Buchter A, Bolt W (1979) Studies on liver microsomal metabolism and interaction of vinyl chloride and related compounds in relation to possible carcinogenicity. Arh Hig Rada Toksikol 30: 369–377

Bolt HM, Filser JG, Buchter A (1981) Inhalation pharmacokinetics based on gas uptake studies III. A pharmacokinetic assessment in man of "peak concentrations" of vinyl chloride. Arch Toxicol 48: 213–228

Bolt HM, Filser JG, Störmer F (1984) Inhalation pharmacokinetics based on gas uptake studies V. Comparative pharmacokinetics of ethylene and 1,3-butadiene in rats. Arch Toxicol 55: 213–218

Buchter A, Bolt HM, Filser JG, Goergens HW, Laib RJ, Bolt W (1978) Pharmakokinetik und Karzinogenese von Vinylchlorid. Arbeitsmedizinische Risikobeurteilung. Verh Dtsch Ges Arbeitsmedizin 18: 111–124

Buchter A, Filser JG, Peter H, Bolt HM (1980) Pharmacokinetics of vinyl chloride in the rhesus monkey. Toxicol Lett 6: 33–36

Csanády GA, Filser JG (1990) SOLVEKIN: a new program for solving pharmaco- and toxicokinetic problems. Presented on the International Workshop on Pharmacokinetic Modelling in Occupational Health, March 4–8, Leysin, Switzerland

Daugherty MS, Ludden TM, Burk RF (1988) Metabolism of ethane and pentane to carbon dioxide by the rat. Drug Metab Dispos 16 [No. 5]: 666–671

Davidson IWF, Parker JC, Beliles RP (1986) Biological basis for extrapolation across mamalian species. Regul Toxicol Pharmacol 6: 211–237

Denk B (1990) Abschätzung des kanzerogenen Risikos von Ethylen und Ethylenoxid für den Menschen durch Speziesextrapolation von der Ratte unter Berücksichtigung der Pharmakokinetik. GSF-Bericht 20/90. Gesellschaft für Strahlen- und Umweltforschung, München

Denk B, Filser JG (1990) Abschätzung des durch Ethylen und Ethylenoxid bedingten kanzerogenen Risikos für den Menschen – Vergleich mit dem Risiko durch endogenes Ethylen. Verh Dtsch Ges Arbeitsmedizin 30 (in press)

Dogra S, Filser JG, Cojocel C, Greim H, Regel U, Oesch F, Robertson LW (1988) Long-term effects of commercial and congeneric polychlorinated biphenyls on ethane production and malonedialdehyde levels, indicators of in vivo lipid peroxidation. Arch Toxicol 62: 369–374

Ehrenberg L, Hiesche KD, Osterman-Golkar S, Wennberg I (1974) Evaluation of genetic risks of alkylating agents: tissue doses in the mouse from air contaminated with ethylene oxide. Mutat Res 24: 83–103

Fernandez J, Guberan E, Caperos J (1976) Experimental human exposure to tetrachloroethylene vapor and elimination in breath after inhalation. Am Ind Hyg Assoc J 37: 143–150

Filser JG, Bolt HM (1979) Pharmacokinetics of halogenated ethylenes in rats. Arch Toxicol 42: 123–136

Filser JG, Bolt HM (1981) Inhalation pharmacokinetics based on gas uptake studies I. Improvement of kinetic models. Arch Toxicol 47: 279–292

Filser JG, Bolt HM (1983) Inhalation pharmacokinetics based on gas uptake studies IV. The endogenous production of volatile compounds. Arch Toxicol 52: 123–133

Filser JG, Bolt HM (1984) Inhalation pharmacokinetics based on gas uptake studies VI. Comparative evaluation of ethylene oxide and butadiene monoxide as exhaled reactive metabolites of ethylene and 1,3-butadiene in rats. Arch Toxicol 55: 219–223

Filser JG, Deml E (1989) Pharmakokinetik und Metabolismus leichtflüchtiger chlorierter Verbindungen am Beispiel von Trichlorethylen, Perchlorethylen und 1,1,1-Trichlorethan (Methylchloroform). VDI Berichte 745: 679–711

Filser JG, Bolt HM, Muliawan H, Kappus H (1983) Quantitative evaluation of ethane and n-pentane as indicators of lipid peroxidation in vivo. Arch Toxicol 52: 135–147

Filser JG, Peter H, Bolt HM, Fedtke N (1987) Pharmacokinetics of the neurotoxin n-hexane in rat and man. Arch Toxicol 60: 77–80

Filser JG, Kessler W, Koch R, Denk B, Summer KH (1988) Kompetitive Hemmung der Bildung des n-Hexan-Metaboliten 2,5-Hexandion durch Toluol: Messung von Pyrrolen im Urin von Ratten. Verh Dtsch Ges Arbeitsmedizin 28: 603–605

Filser JG, Denk B, Törnqvist M, Kessler W, Ehrenberg L (1991) Pharmacokinetics of ethylene in man; body burden with ethylene oxide and hydroxyethylation of hemoglobin due to endogenous and environmental ethylene. Arch Toxicol, submitted for publication

Fiserova-Bergerova V, Teisinger J (1965) Pulmonary styrene vapor retention. Industrial Medicine and Surgery 34 [No 8]: 620–622

Fisher JW, Whittaker TA, Taylor DH, Clewell HJ III, Andersen ME (1989) Physiologically based pharmacokinetic modeling of the pregnant rat: a multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. Toxicol Appl Pharmacol 99: 395–414

Frank H, Dürk H (1983) Determination of alkanes in breath to monitor lipid peroxidation in the presence of volatile toxicants and metabolites. An optimized automatic method. Arch Toxicol 53: 213–223

Frank H, Hintze T, Bimboes D, Remmer H (1980) Monitoring lipid peroxidation by breath analysis: endogenous hydrocarbons and their metabolic elimination. Toxicol Appl Pharmacol 56: 337–344

Freundt KJ, Römer KG, Federsel RJ (1989) Decrease of inhaled toluene, ethyl benzene, m-xylene, or mesitylene in rat blood after combined exposure to ethyl acetate. Bull Environ Contam Toxicol 42: 495–498

Gargas ML, Andersen ME, Clewell HJ III (1986) A physiologically-based simulation approach for determining metabolic constants from gas uptake data. Toxicol Appl Pharmacol 86: 341–352

Gargas ML, Clewell HJ III, Andersen ME (1990) Gas uptake inhalation techniques and the rates of metabolism of chloromethanes, chloroethanes, and chloroethylenes in the rat. Inhalation Toxicology 2: 295–319

Garman RH, Snellings WM, Maronpot RR (1984) Brain tumors in F344 rats associated with chronic inhalation exposure to ethylene oxide. Neurotoxicology 6: 117–138

Golka K, Peter H, Denk B, Filser JG (1989) Pharmacokinetics of propylene and its reactive metabolite propylene oxide in Sprague-Dawley rats. Arch Toxicol Suppl 13: 240–242

Guyton AC (1947) Respiratory volumes of laboratory animals. Am J Physiol 150: 70–77

Hartmann M, Kessler W, Denk B, Filser JG (1990) Pharmakokinetik und endogene Produktion von Isopren beim Menschen. Verh Dtsch Ges Arbeitsmedizin 30 (in press)

Hallier E, Filser JG, Bolt HM (1981) Inhalation pharmacokinetics based on gas uptake studies II. Pharmacokinetics of acetone in rats. Arch Toxicol 47: 293–304

Hefner RE, Watanabe PG, Gehring PJ (1975) Preliminary studies on the fate of inhaled vinyl chloride monomer in rats. Ann NY Acad Sci 246: 135–148

Igwe OJ, Que Hee SS, Wagner WD (1986) Inhalation pharmacokinetics of 1,2-dichloroethane after different dietary pretreatment of male Sprague-Dawley rats. Arch Toxicol 59: 127–134

Johanson G (1986) Physiologically based pharmacokinetic modeling of inhaled 2-butoxyethanol in man. Toxicol Lett 34: 23–31

Kappus H, Muliawan H (1982) Alkane formation during liver microsomal lipid peroxidation. Biochem Pharmacol 31: 597–600

Kappus H, Bolt HM, Buchter A, Bolt W (1976) Liver microsomal uptake of [^{14}C] vinyl chloride and transformation to protein alkylating metabolites in vitro. Toxicol Appl Pharmacol 37: 461–471

Kessler W, Denk B, Filser JG (1989) Species-specific inhalation pharmacokinetics of 2-nitropropane, methyl ethyl ketone, and n-hexane. In: Travic CC (ed) Biologically-based methods for cancer risk assessment. Plenum Publishing Corporation, New York and London, pp 123–139

Kessler W, Heilmaier H, Kreuzer P, Shen JH, Filser M, Filser JG (1990) Spectrophotometric determination of pyrrole-like substances in urine of rat and man: an assay for the evaluation of 2,5-hexanedione formed from n-hexane. Arch Toxicol 64: 242–246

Kivits GAA, Ganguli-Swarttouw MACR, Christ EJ (1981) The composition of alkanes in exhaled air of rats as a result of lipid peroxidation in vivo. Biochim Biophys Acta 665: 559–570

Kreiling R, Laib RJ, Filser JG, Bolt HM (1986) Species differences in butadiene metabolism between mice and rats evaluated by inhalation pharmacokinetics. Arch Toxicol 58: 235–238

Kreiling R, Laib RJ, Filser JG, Bolt HM (1987) Inhalation pharmacokinetics of 1,2-epoxybutene-3 reveal species differences between rats and mice sensitive to butadiene-induced carcinogenesis. Arch Toxicol 61: 7–11

Kreuzer PE, Kessler W, Welter HF, Baur C, Filser JG (1991) Enzyme specific kinetics of 1,2-epoxybutene-3 in microsomes and cytosol from livers of mouse, rat, and man. Arch Toxicol 65: 59–67

Liira J, Johanson G, Riihimäki V (1990) Dose-dependent kinetics of inhaled methylethylketone in man. Toxicol Lett 50: 195–201

Link B (1979) Metabolismus von Perchloräthylen in vivo und in vitro. Diplomarbeit an der Fakultät für Chemie und Pharmazie der Universität Tübingen

Monster AC, Boersma G, Duba WC (1976) Pharmacokinetics of trichloroethylene in volunteers, influence of workload and exposure concentration. Int Arch Occup Environ Hlth 38: 87–102

Muliawan H, Scheulen ME, Kappus H (1982) Adriamycin stimulates only the iron ion-induced, NADPH-dependent microsomal alkane formation. Biochem Pharmacol 31: 3147–3150

Nelder JA, Mead R (1965) A simplex method for function minimization. Comp J 7: 308–313

Nolan RJ, Freshour NL, Rick DL, McCarty LP, Saunders JH (1984) Kinetics and metabolism of inhaled methyl chloroform (1,1,1-trichloroethane) in male volunteers. Fundam Appl Toxicol 4: 654–662

Nomiyama K, Nomiyama H (1974) Respiratory retention, uptake and excretion of organic solvents in man. Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate, ethyl alcohol. Int Arch Arbeitsmed 32: 75–83

Ottenwälder H, Kappus H, Bolt HM (1983) Covalent protein binding of vinyl chloride metabolites during co-incubation of freshly isolated hepatocytes and hepatic sinusoidal cells of rats. Arch Toxicol Suppl 6: 266–270

Peter H, Bolt HM (1984) Experimental pharmacokinetics and toxicology of acrylonitrile. G Ital Med Lav 6: 77–81

Peter H, Filser JG, v. Szentpály L, Wiegand HJ (1986) Different pharmacokinetics of dichlorofluoromethane (CFC21) and chlorodifluoromethane (CFC 22). Arch Toxicol 58: 282–283

Peter H, Wiegand HJ, Bolt HM, Greim H, Walter G, Berg M, Filser JG (1987) Pharmacokinetics of isoprene in mice and rats. Toxicol Lett 36: 9–14

Prout MS, Provan WM, Green T (1985) Species differences in response to trichloroethylene. I. Pharmacokinetics in rats and mice. Toxicol Appl Pharmacol 79: 389–400

Remmer H, El Majid Gharaibeh A (1984) Measurement of the oxidation rate of volatile alkanes: a new and non-invasive procedure for testing the drug-metabolizing capacity of the liver. Biochem Soc Trans 12: 28–30

Robertson LW, Regel U, Filser JG, Oesch F (1985) Absence of lipid peroxidation as determined by ethane exhalation in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Arch Toxicol 57: 13–16

Sato A, Nakajima T (1979 a) A vial-equilibration method to evaluate the drug-metabolizing enzyme activity for volatile hydrocarbons. Toxicol Appl Pharmacol 47: 41–46

Sato A, Nakajima T (1979 b) A structure-activity relationship of some chlorinated hydrocarbons. Arch Environ Health Mar./Apr.: 69–75

Schwegler U, Jiang X, Kessler W, Johanson G, Filser JG (1990) Pharmakokinetik von Styrol bei Ratte und Maus und Bestimmung von Styrol-7,8-oxid im Blut von Ratten. Verh Dtsch Ges Arbeitsmedizin 30 (in press)

Shen J, Kessler W, Denk B, Filser JG (1989) Metabolism and endogenous production of ethylene in rat and man. Arch Toxicol Suppl 13: 237–239

Siegers C-P, Filser JG, Bolt HM (1978) Effect of dithiocarb on metabolism and covalent binding of carbon tetrachloride. Toxicol Appl Pharmacol 46: 709–716

Simon P, Filser JG, Bolt HM (1985) Metabolism and pharmacokinetics of vinyl acetate. Arch Toxicol 57: 191–195

Snellings WM, Weil CS, Maronpot RR (1984) A two-year inhalation study of the carcinogenic potential of ethylene oxide in Fischer 344 rats. Toxicol Appl Pharmacol 75: 105–117

Stacey NH, Kappus H (1982) Cellular toxicity and lipid peroxidation in response to mercury. Toxicol Appl Pharmacol 63: 29–35

Stewart RD, Hugh C, Dodd AB, Baretta ED, Schaffer AW (1968) Human exposure to styrene vapor. Arch Environ Health 16: 656–662

Wigaeus E, Holm S, Åstrand I (1981) Exposure to acetone. Uptake and elimination in man. Scand J Work Environ Health 7: 84–94