

Inhalative Exposition

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Overview

- Introduction
- Developing a physiological toxicokinetic model for isoprene
- Summary

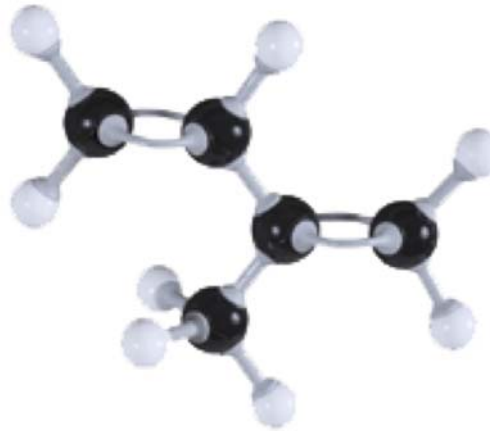
Daily supply for an average adult

The importance of inhalation exposure becomes clear when comparing the daily supply of water, food and air for an average adult:

Water	1.9-2.6 l
Food	1.4-1.7 kg
Air (inhaled)	21-24 m ³ or 24-29 kg

Data from Report of the Task Group on Reference Man (1975)

Example: isoprene



- Volatile liquid

Boiling point 34 °C

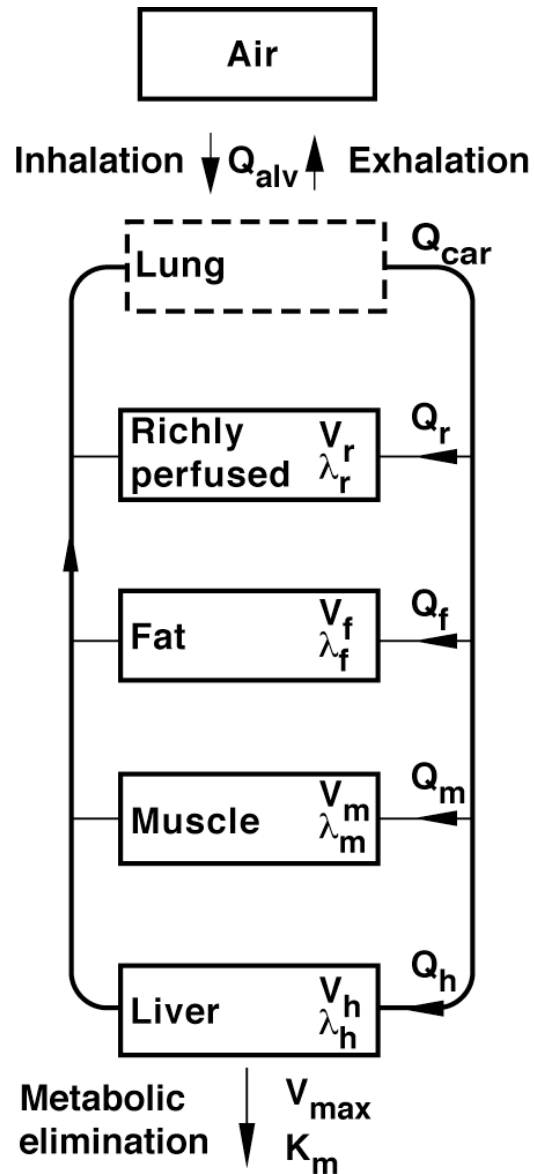
- Ubiquitous chemical

Major endogenously produced hydrocarbon in humans

Annual emission by plants: 500 million tons

Formation during combustion processes (automobile exhaust, tobacco smoke)

Physiological toxicokinetic model I

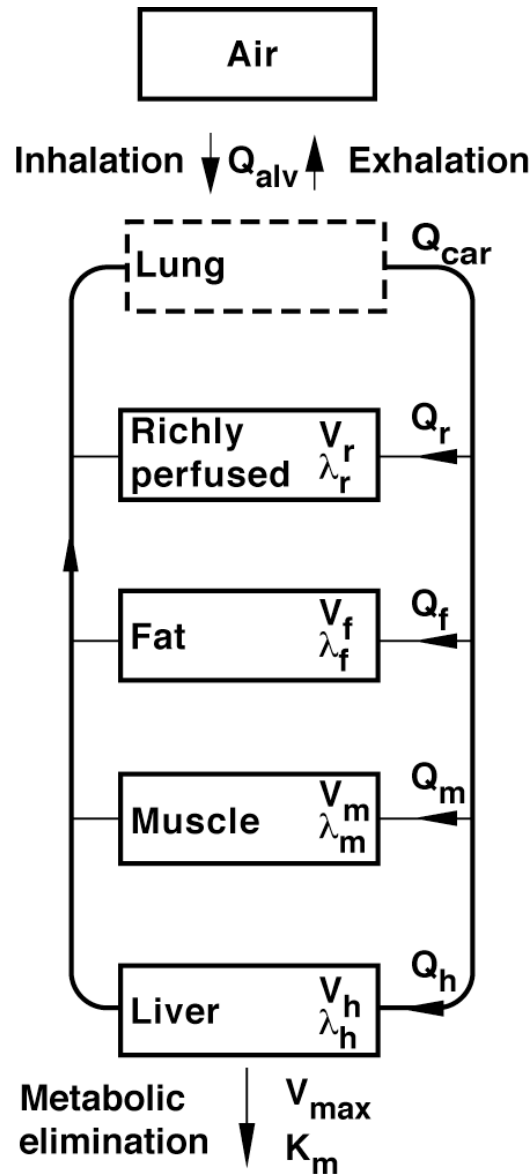


Integrative approach:

- comprises organs and tissues integrated via the vascular system (biology)
- considers diffusion, tissue affinity, protein binding (physico-chemistry)
- accounts for enzyme activity (biochemistry)

This approach has the potential to make species extrapolations.

Mathematical representation of the model I



$$V_{Cham} * \frac{dC_{exp}}{dt} = Q_{alv} * \left(\frac{C_{art}}{\lambda_{blood:air}} - C_{exp} \right), C_{exp}(0) = C_0$$

$$C_{art} = \frac{Q_{car} * \sum Q_i * C_{vi} / \sum Q_i + Q_{alv} * C_{exp}}{Q_{car} + \frac{Q_{alv}}{\lambda_{blood:air}}}$$

$$V_r * \frac{dC_r}{dt} = Q_r * \left(C_{art} - \frac{C_r}{\lambda_r} \right), C_r(0) = 0$$

$$V_f * \frac{dC_f}{dt} = Q_f * \left(C_{art} - \frac{C_f}{\lambda_f} \right), C_f(0) = 0$$

$$V_m * \frac{dC_m}{dt} = Q_m * \left(C_{art} - \frac{C_m}{\lambda_m} \right), C_m(0) = 0$$

$$V_h * \frac{dC_h}{dt} = Q_h * \left(C_{art} - \frac{C_h}{\lambda_h} \right) - \frac{V_{max} * C_h}{K_m + C_h}, C_h(0) = 0$$

Data required for model development

- Physiological data

Tissue volumes, blood flows and alveolar ventilation

- In-vitro data

Partition coefficients tissue:blood and blood:air

- In-vivo data

Closed chamber data

Exposure at steady state

Physiological parameters

Parameter	Mouse	Rat	Human
Body weight (kg)	0.025	0.25	70
Alveolar ventilation (l/h)	0.90*	4.2*	300
Cardiac output (l/h)	1.02	5.0	372
Organ volumes (%)			
Liver	5.5	4	2.6
Fat	10	7	19
Richly perfused tissues	5	5	5
Slowly perfused tissues	70	75	62
Organ blood flows (%)			
Liver	25	25	26
Fat	9	9	5
Richly perfused tissues	51	51	44
Slowly perfused tissues	15	15	25

“Reference values” from Arms and Travis (1988)

* reduced to 60% of the reference value

Partition coefficients of isoprene

The partition coefficient (λ) is the ratio of the concentrations of a compound between two phases at equilibrium. It is a measure for enrichment.

Partition coefficient	Mouse	Rat	Human
Blood:air	2.04	2.33	0.75
Fat:blood	30.2	26.4	82
Muscle:blood	0.73	0.64	1.97
Liver:blood	0.95	0.83	2.57
Richly perfused tissues:blood*	0.91	0.79	2.45

*Mean of liver:blood and kidney:blood

Data from Filser et al. (1996)

Closed chamber system

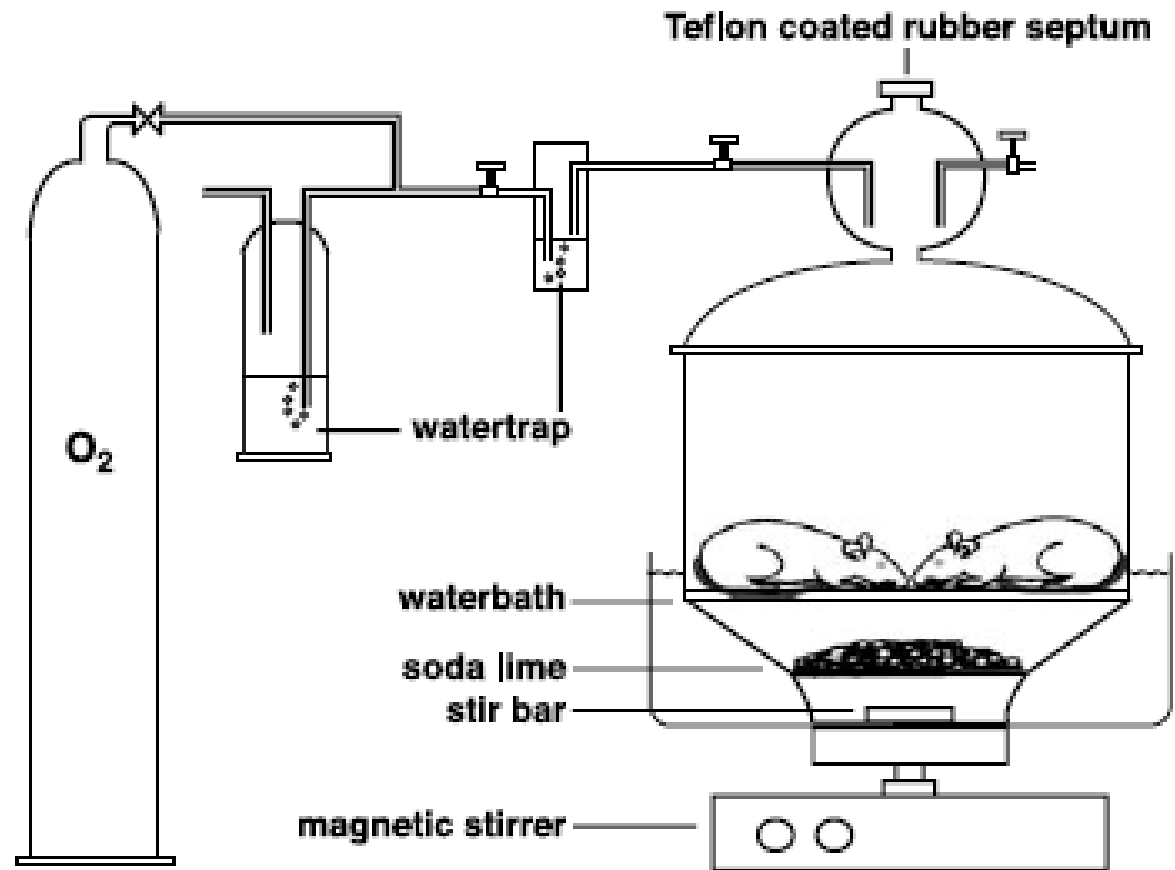
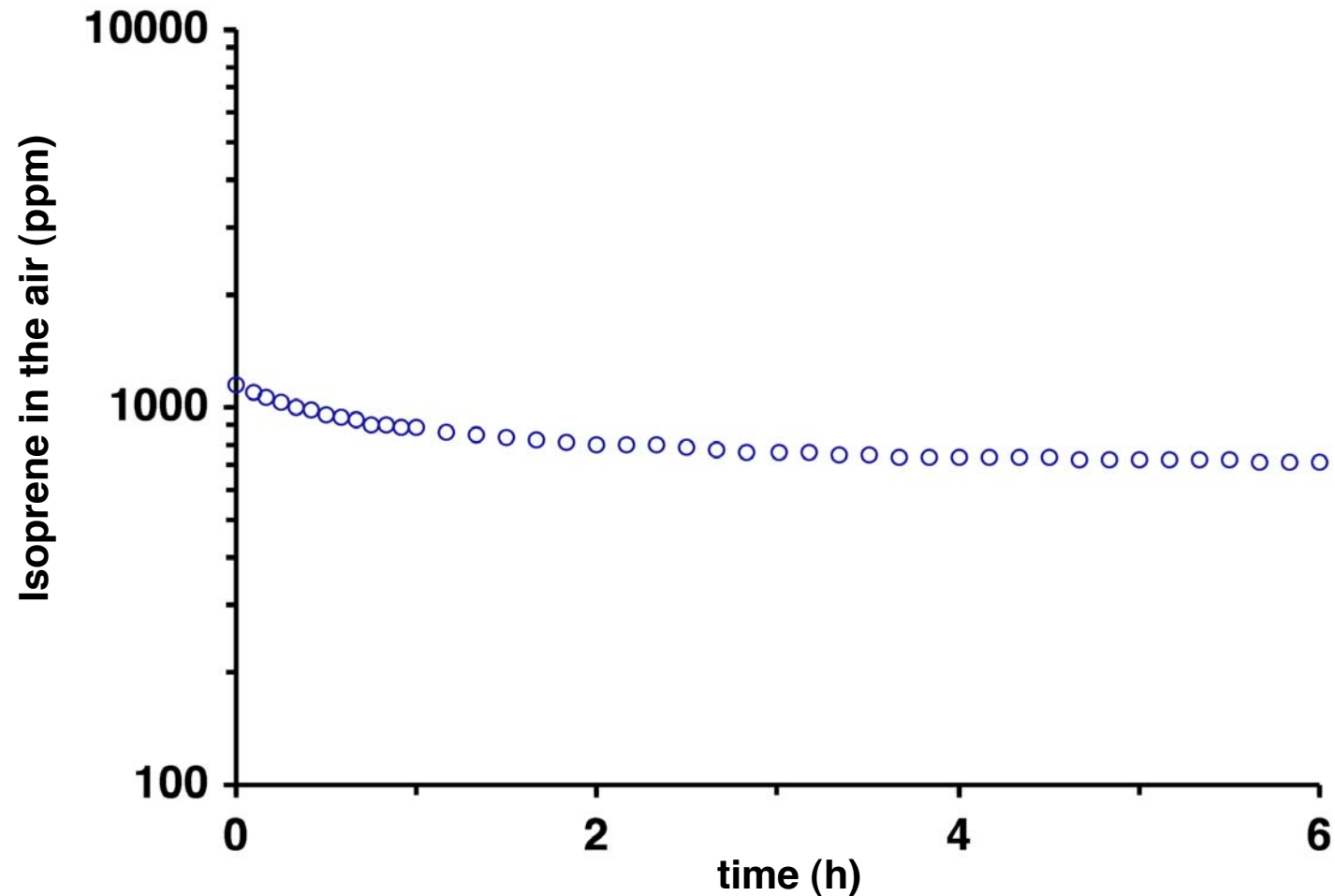


Figure 2. The “Tuebingen Desiccator,” an all-glass closed chamber system (CCS) for studying the fate of endogenous and exogenous volatile organic compounds in rodents.

Inhalation experiments with isoprene I

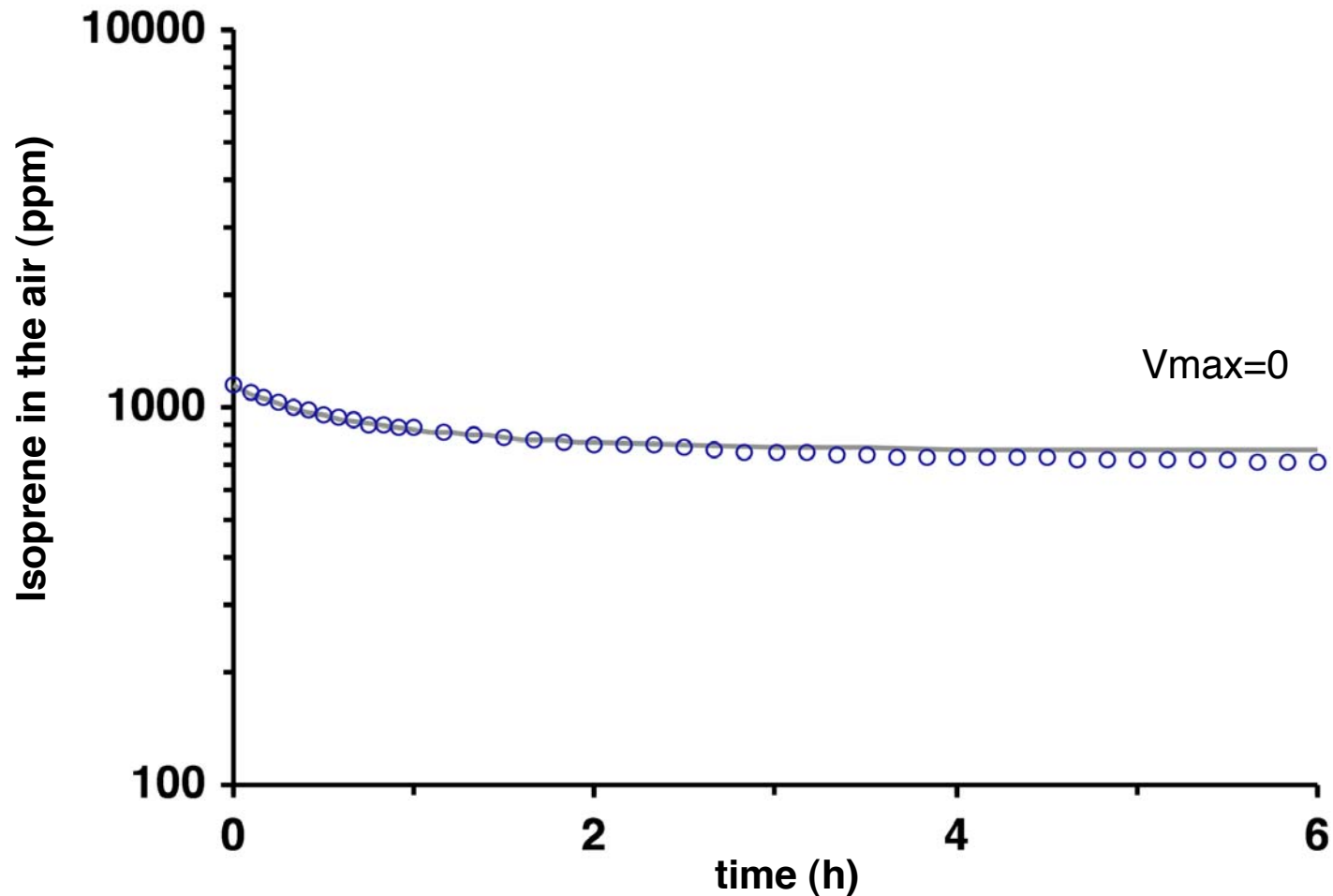
Concentration-time course of inhaled isoprene in a closed exposure chamber containing two male Wistar rats following pretreatment with diethyldithiocarbamate



Data from Peter et al (1987)

Inhalation experiments with isoprene I

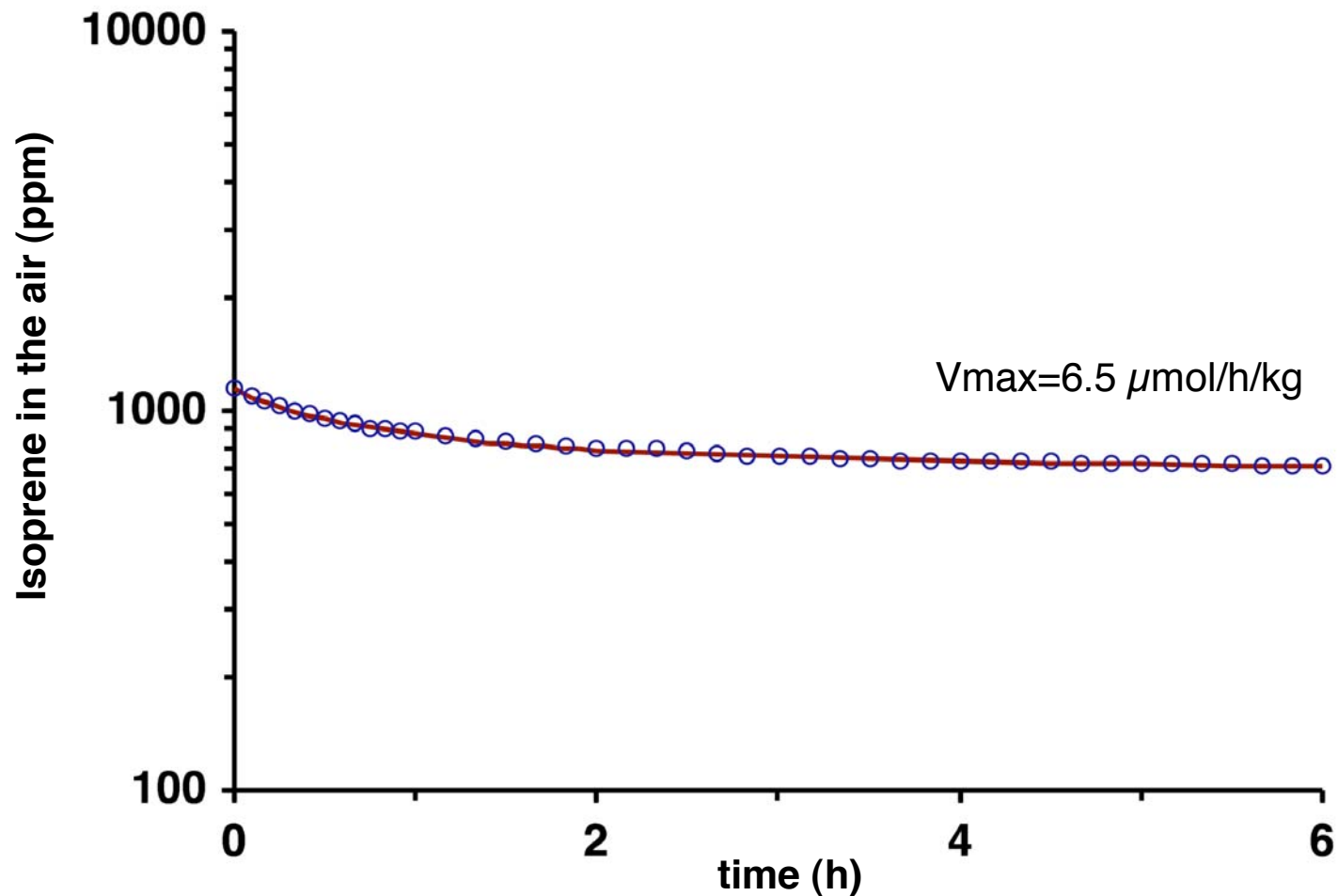
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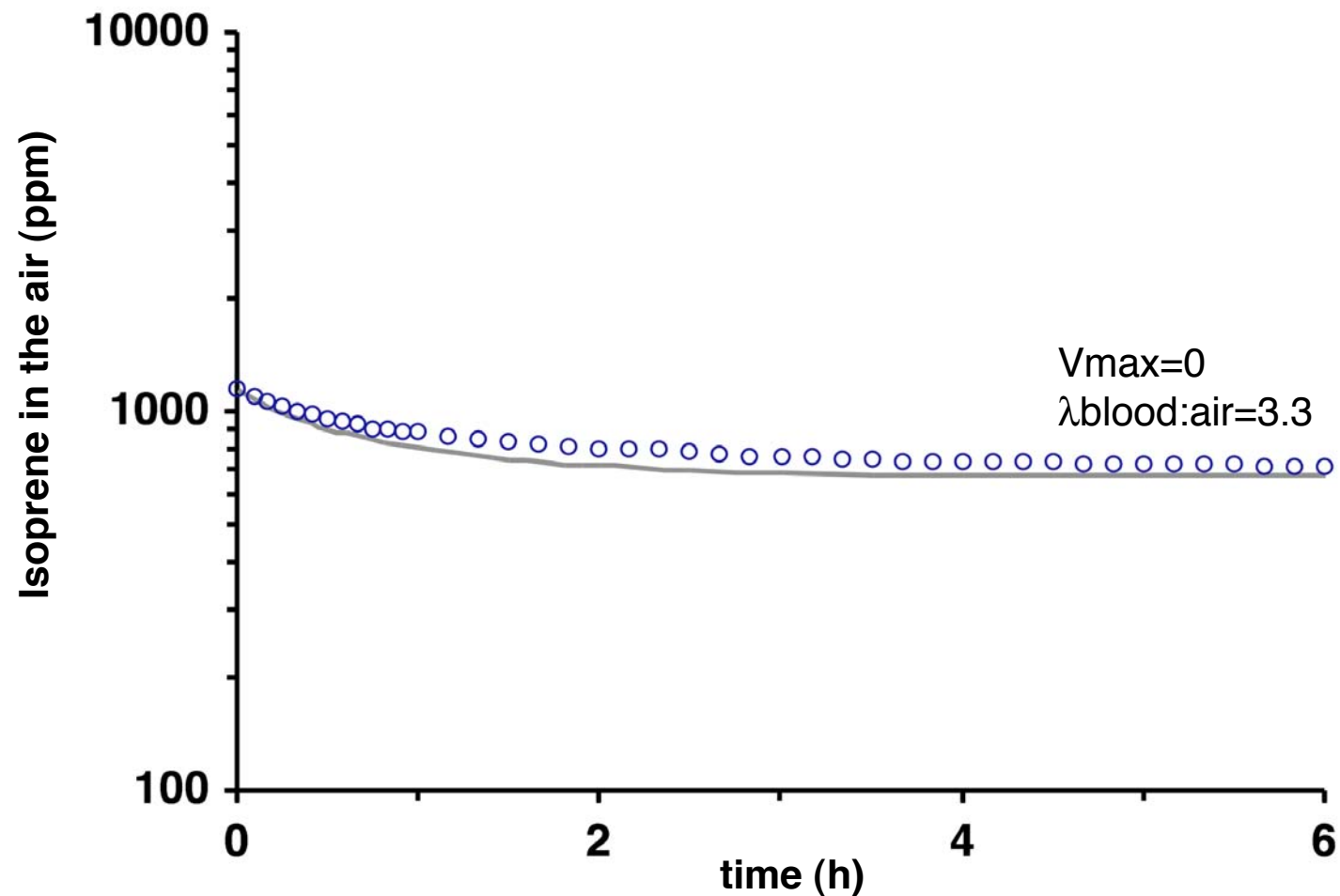
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Inhalation experiments with isoprene I

In-vivo data under the condition of metabolic inhibition is very useful:

- to inspect the process of distribution
- to verify the values of the partition coefficient(s)
- to check the values of the underlying physiological parameters

Inhalation experiments with isoprene II

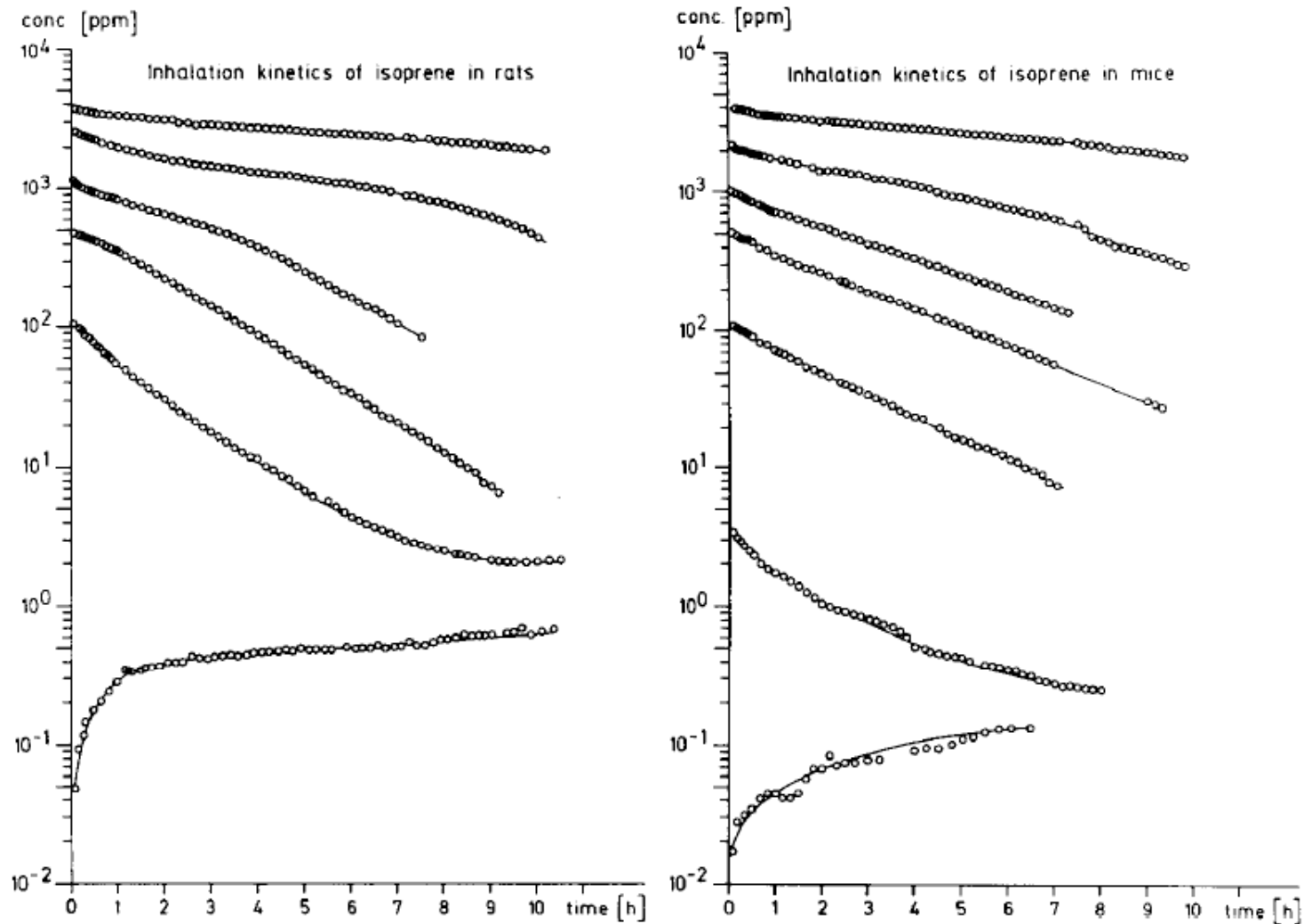
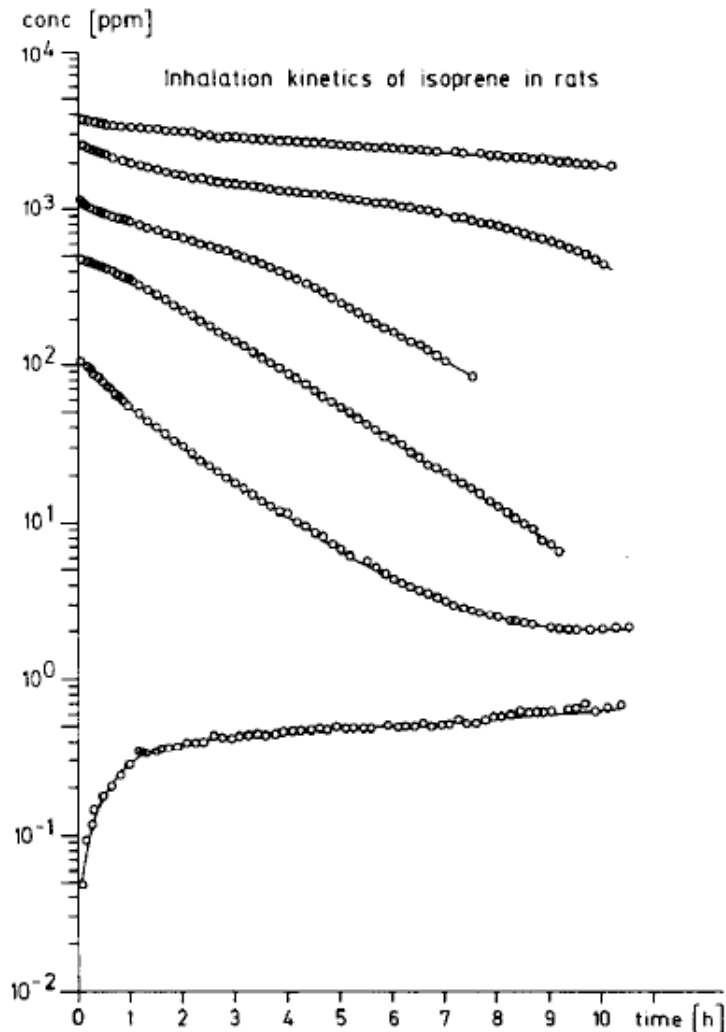


Fig. 1. Concentration-time curves of isoprene in closed exposure systems of 6.4-liter volume occupied by 2 rats and 5 mice, respectively, in each experiment. Open circles, measured values; solid lines, graphical extrapolation.

Inhalation experiments with isoprene II

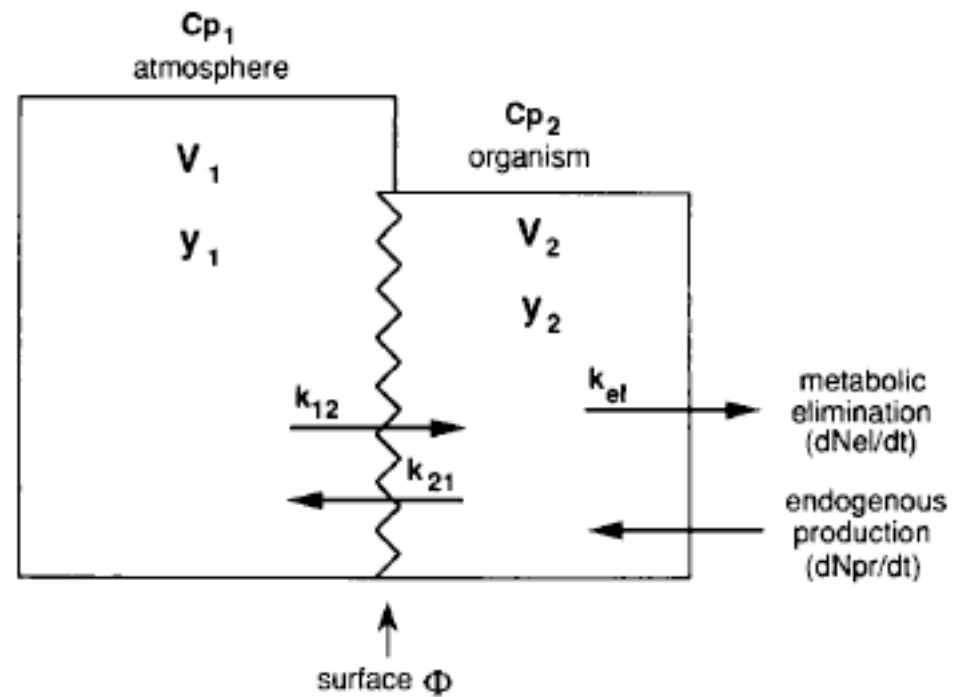


The slopes decline with increasing initial exposure concentration indicating saturation of the metabolic elimination.

An endogenous production is apparent which is related to the formation of acetone instead of isoprene (Filser et al. 1996).

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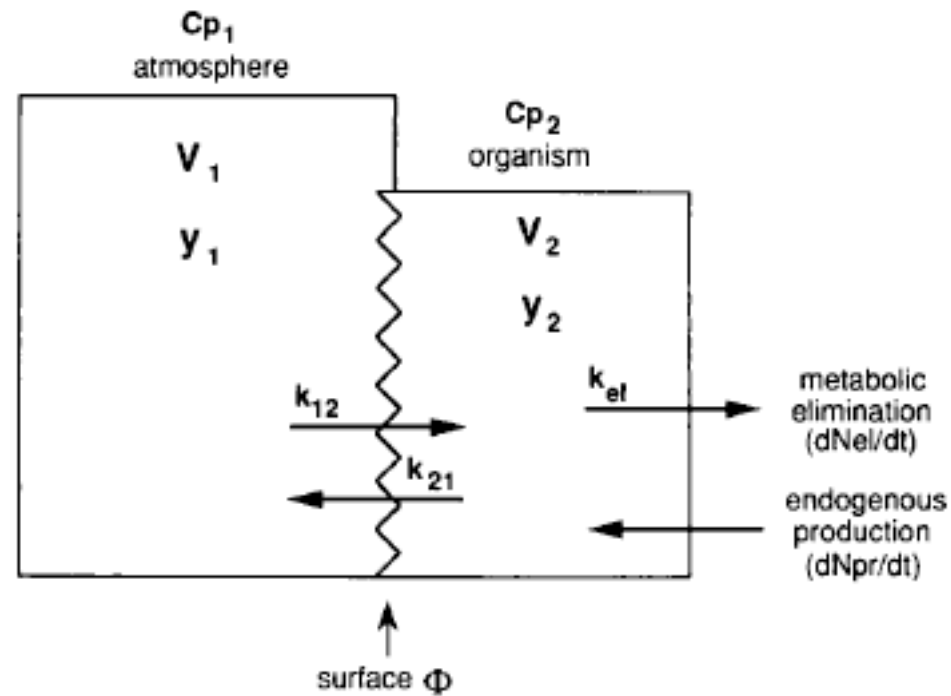
First toxicokinetic analysis of isoprene data



Filser et al (1992)

Fig. 4. Pharmacokinetic two compartment model for the closed exposure system.

First toxicokinetic analysis of isoprene data



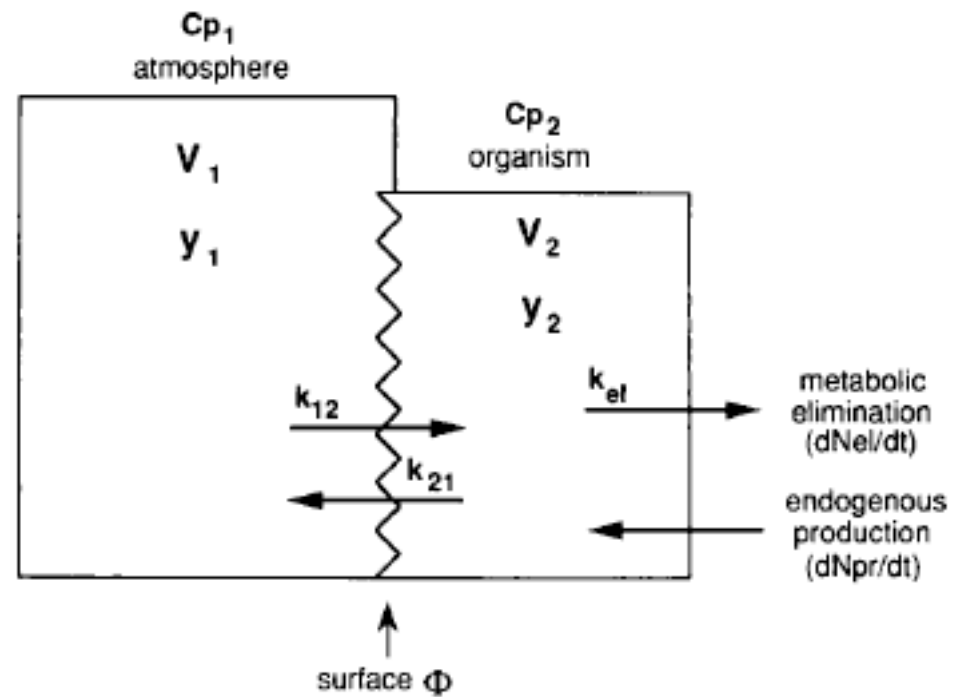
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Fig. 4. Pharmacokinetic two compartment model for the closed exposure system.

Parameter r	Mouse	Rat
V_{max} ($\mu\text{mol/h/kg}$)	410	110
K_m (mmol/l)	0.06	0.026

Parameters from Filser et al (1996)

First toxicokinetic analysis of isoprene data



Filser et al (1992)

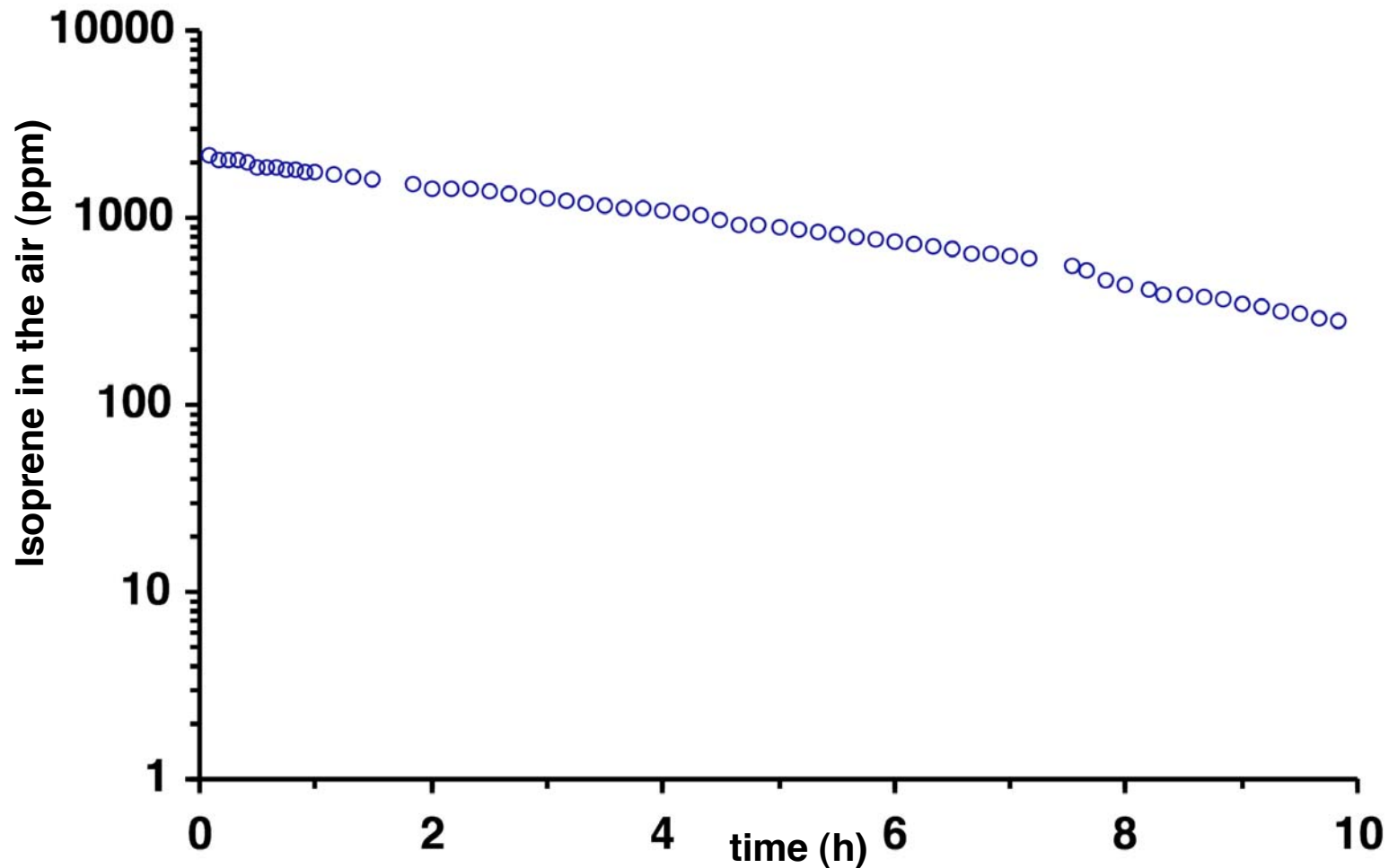
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Parameter	Mouse	Rat
Vmax ($\mu\text{mol/h/kg}$)	410	110
Km (mmol/l)	0.06	0.026
KmPT (mmol/l)	0.004	0.002

Parameters from Filser et al (1996)

Inhalation experiments with isoprene II

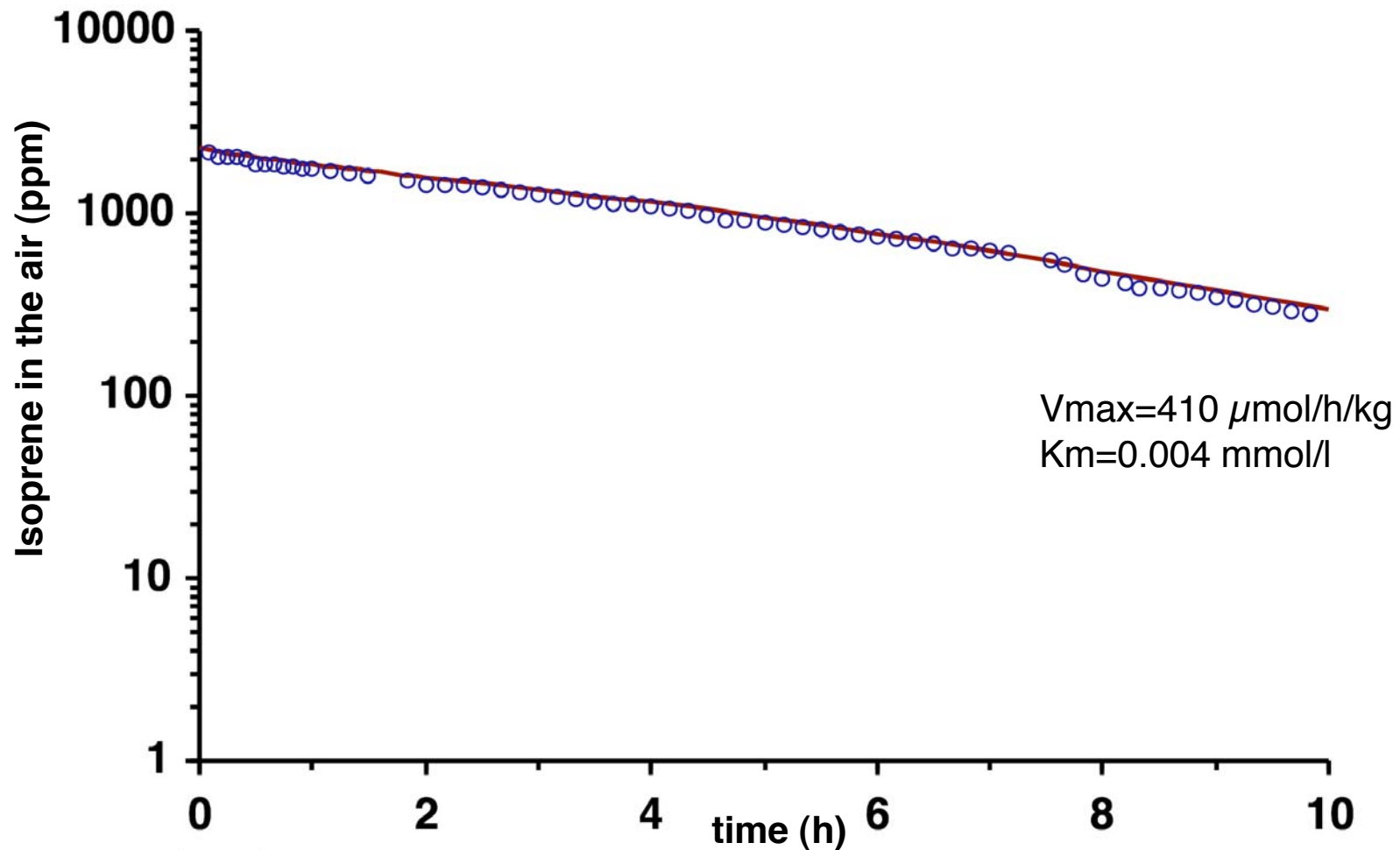
Concentration-time course of inhaled isoprene in a closed exposure chamber containing five male B6C3F1 mice



Data from Peter et al (1987)

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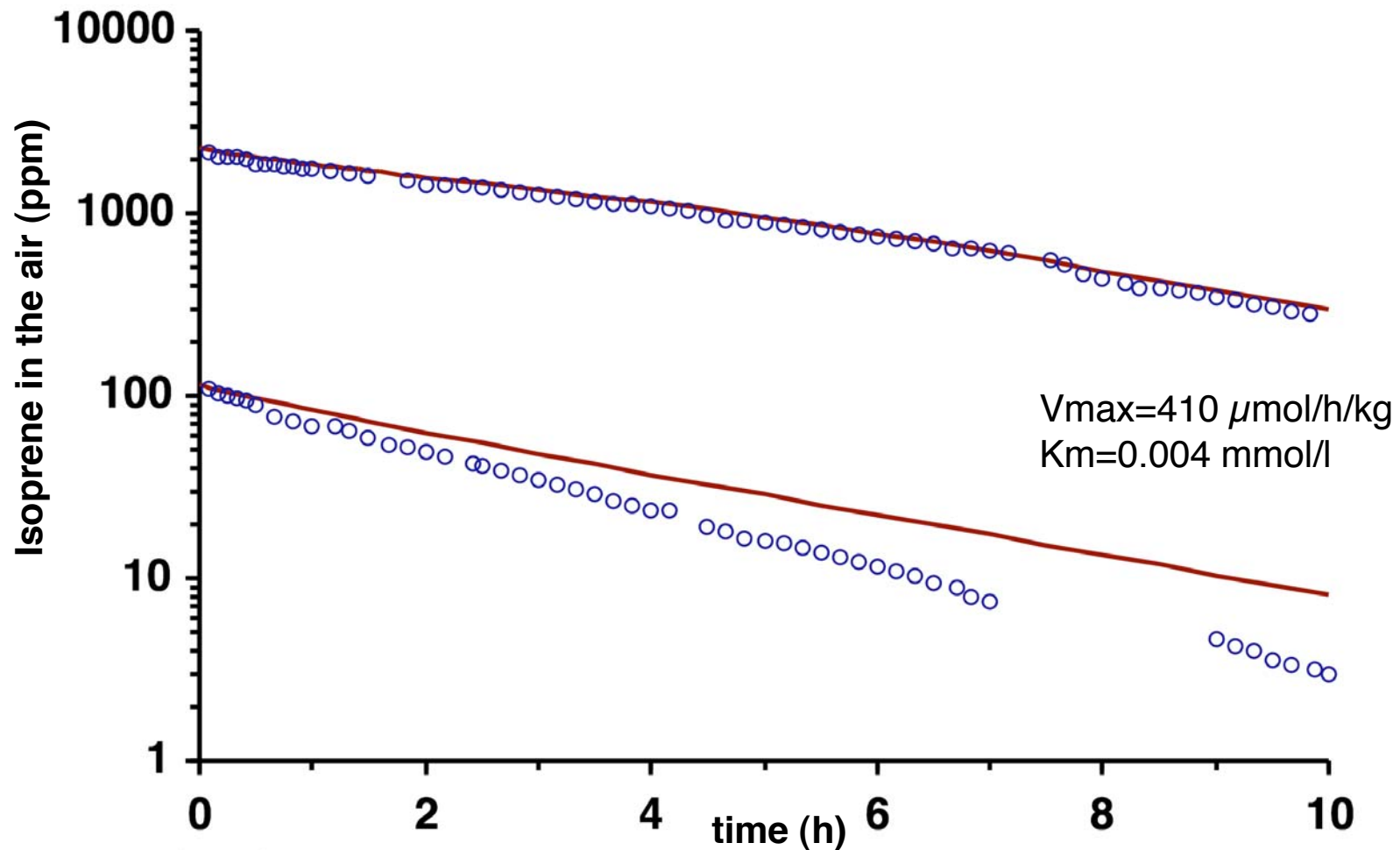
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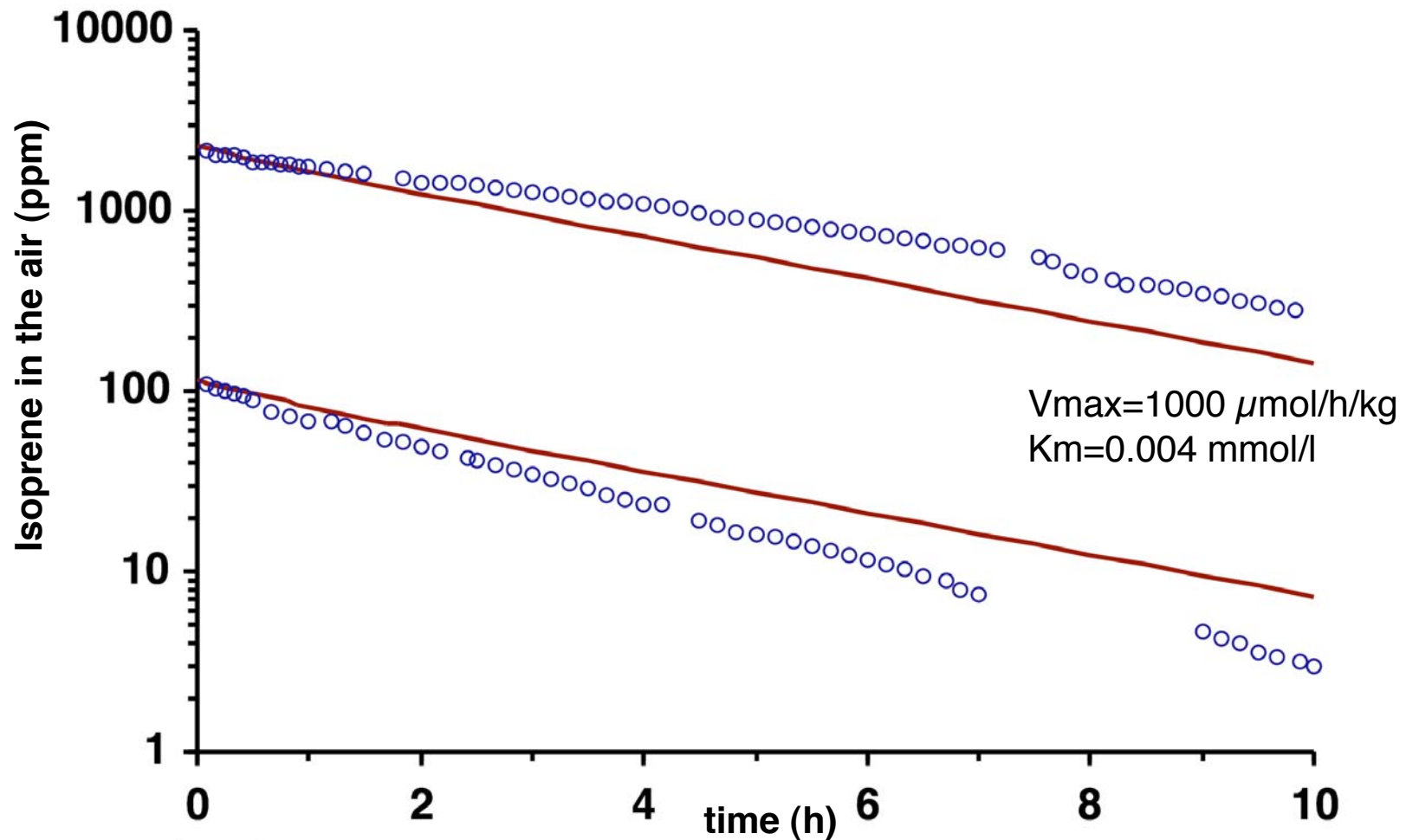
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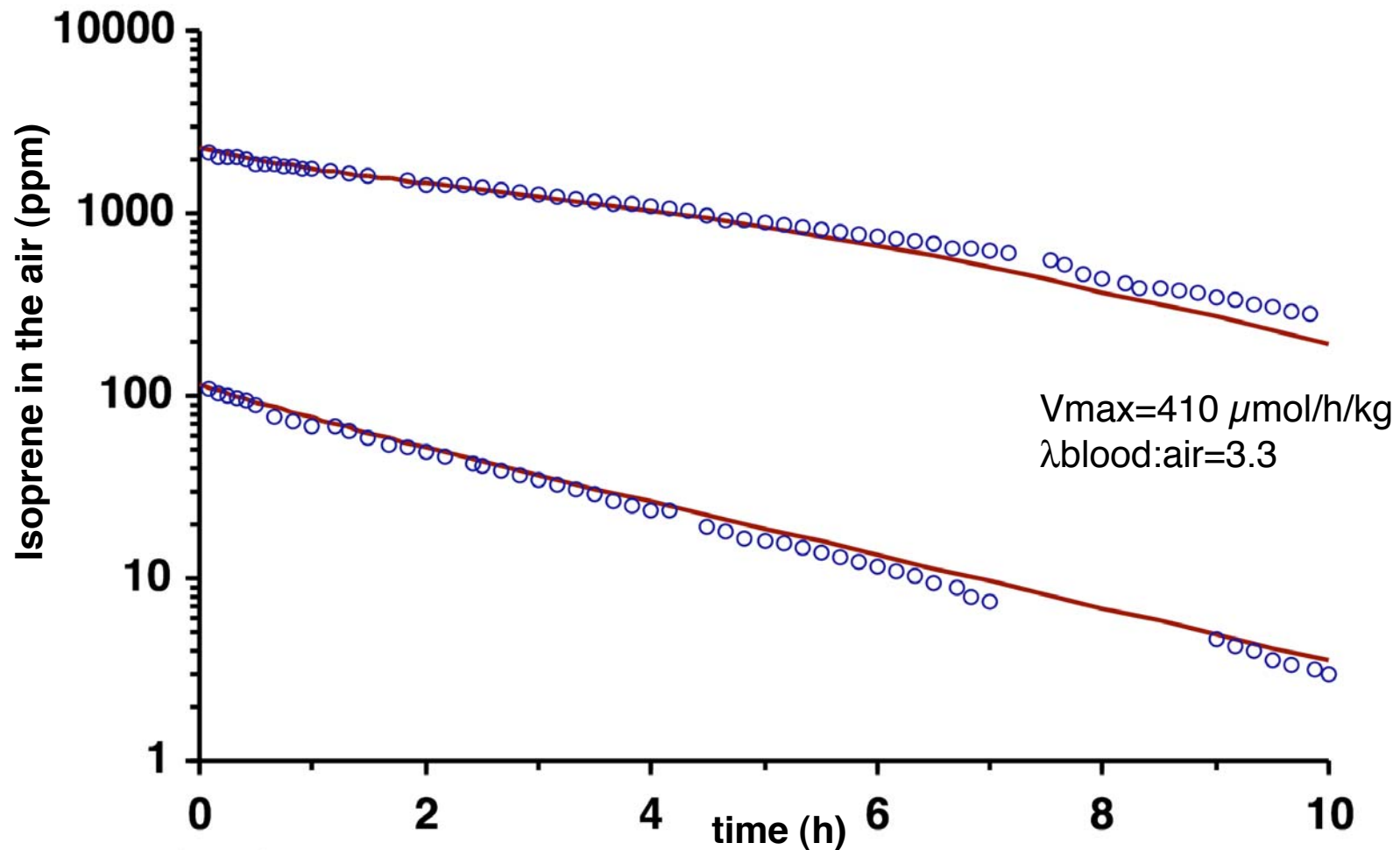
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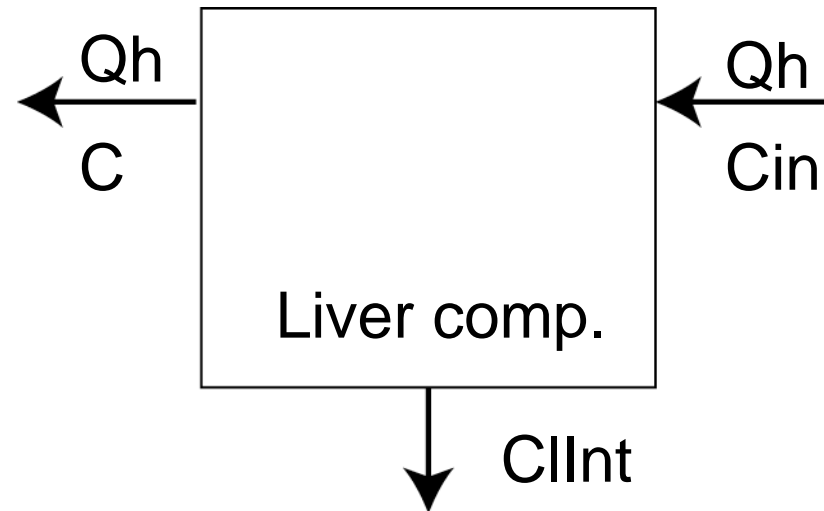
Inhalation experiments with isoprene II

Concentration-time curves of inhaled isoprene in a closed exposure chamber containing five male B6C3F1 mice



Data from Peter et al (1987)

Perfusion limited metabolism in the liver

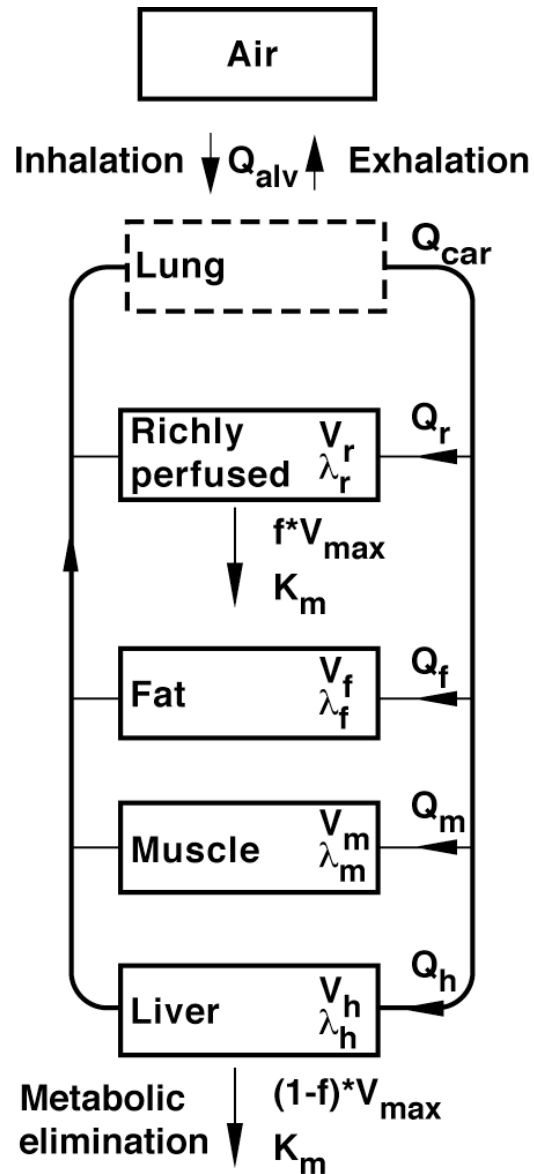


Blood flow through the liver limits the liver clearance even though the intrinsic metabolic clearance could be much higher.

$$\text{The liver clearance: } Cl_H = \frac{C_{in} - C}{C_{in}} * Q_h = \frac{Q_h * Cl_{Int}}{Q_h + Cl_{Int}} \quad \text{Wilkinson and Shand (1975)}$$

$$\text{If } \lim_{Cl_{Int} \rightarrow \infty} Cl_H \rightarrow Q_h$$

Model improvement I

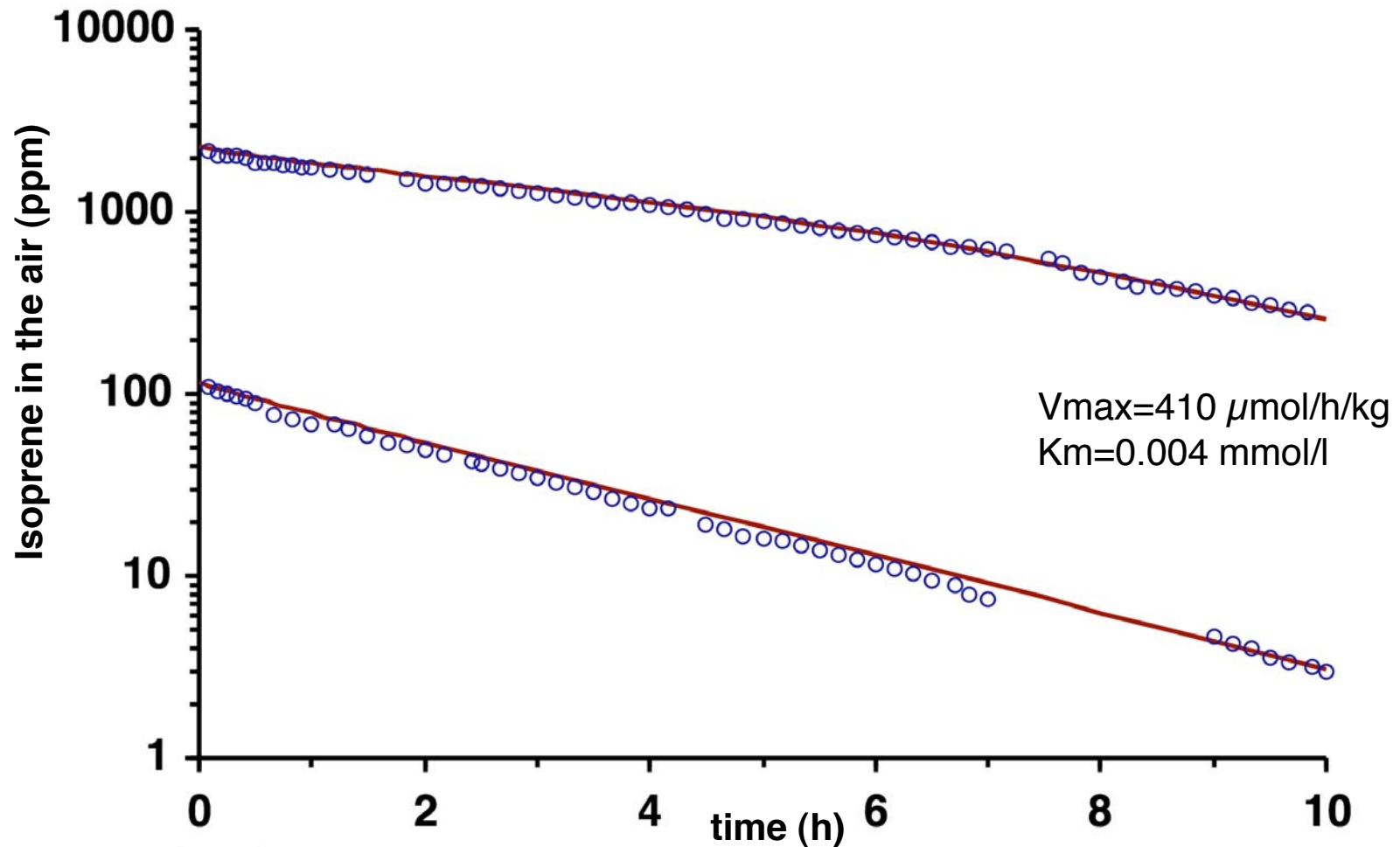


About 10% of total mixed function mono-oxygenases are located in extrahepatic tissues ($f=0.1$).

Additional metabolic capacity is located in the richly perfused tissue group (lung, kidney, brain).

Inhalation experiments with isoprene II

Concentration-time curves of inhaled isoprene in a closed exposure chamber containing five male B6C3F1 mice



Data from Peter et al (1987)

Inhalation experiments with isoprene II

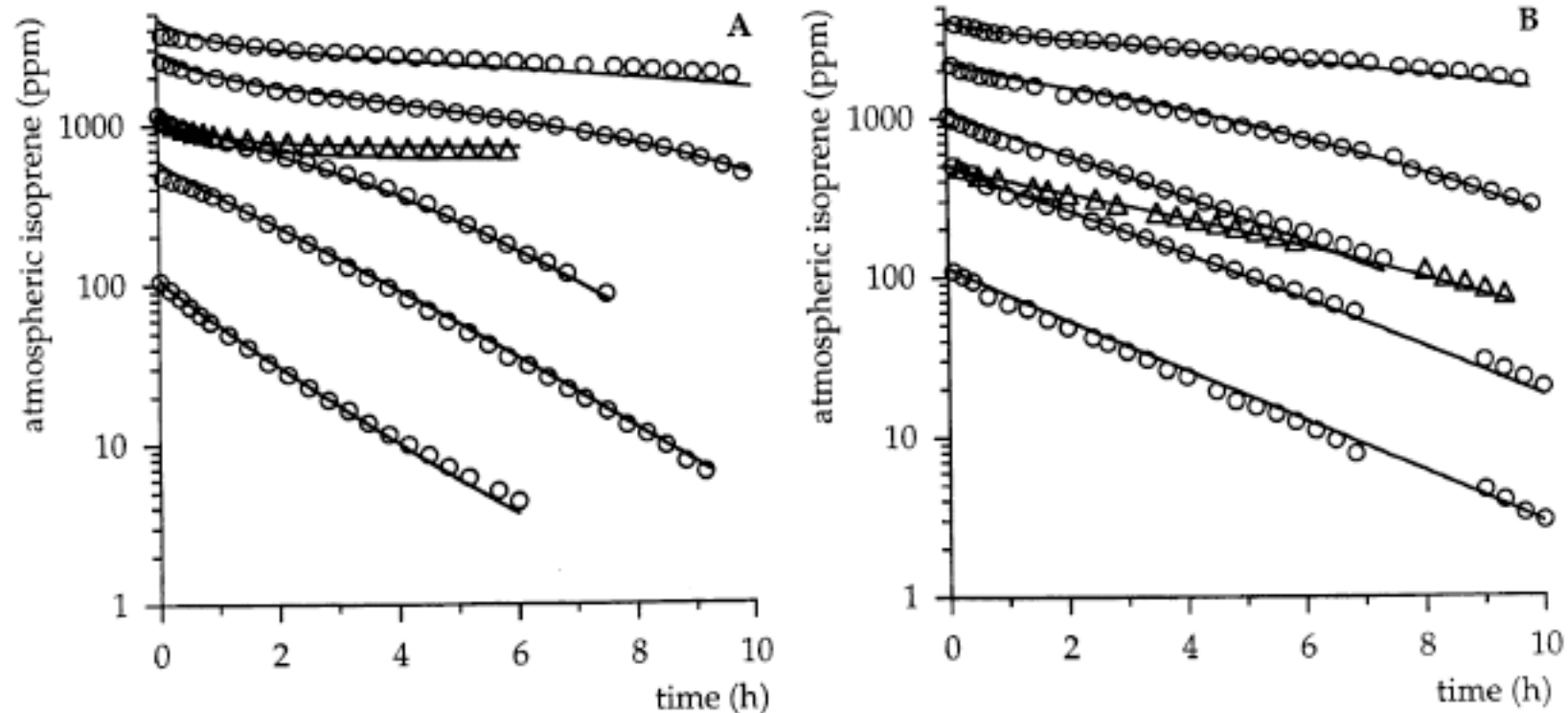


Fig. 1. Concentration–time curves of inhaled IP in closed exposure chambers containing two male Wistar rats (A) or five male B6C3F₁ mice (B). Symbols represent data points [13] measured in diethyldithiocarbamate pretreated (Δ) and in naïve animals (\circ). The solid lines are predictions by the PT-model.

Filser et al (1996) and Csanády et al. (2001)

Inhalation experiments with isoprene II

In-vivo data collected over a broad concentration range and time span are useful:

- to inspect the type of metabolic elimination (saturation vs linear kinetics)
- to observe an eventual perfusion limited metabolic elimination
- to determine the numerical value of metabolic parameter(s)
- to make species comparisons if possible

Modified spirometer system for human exposure

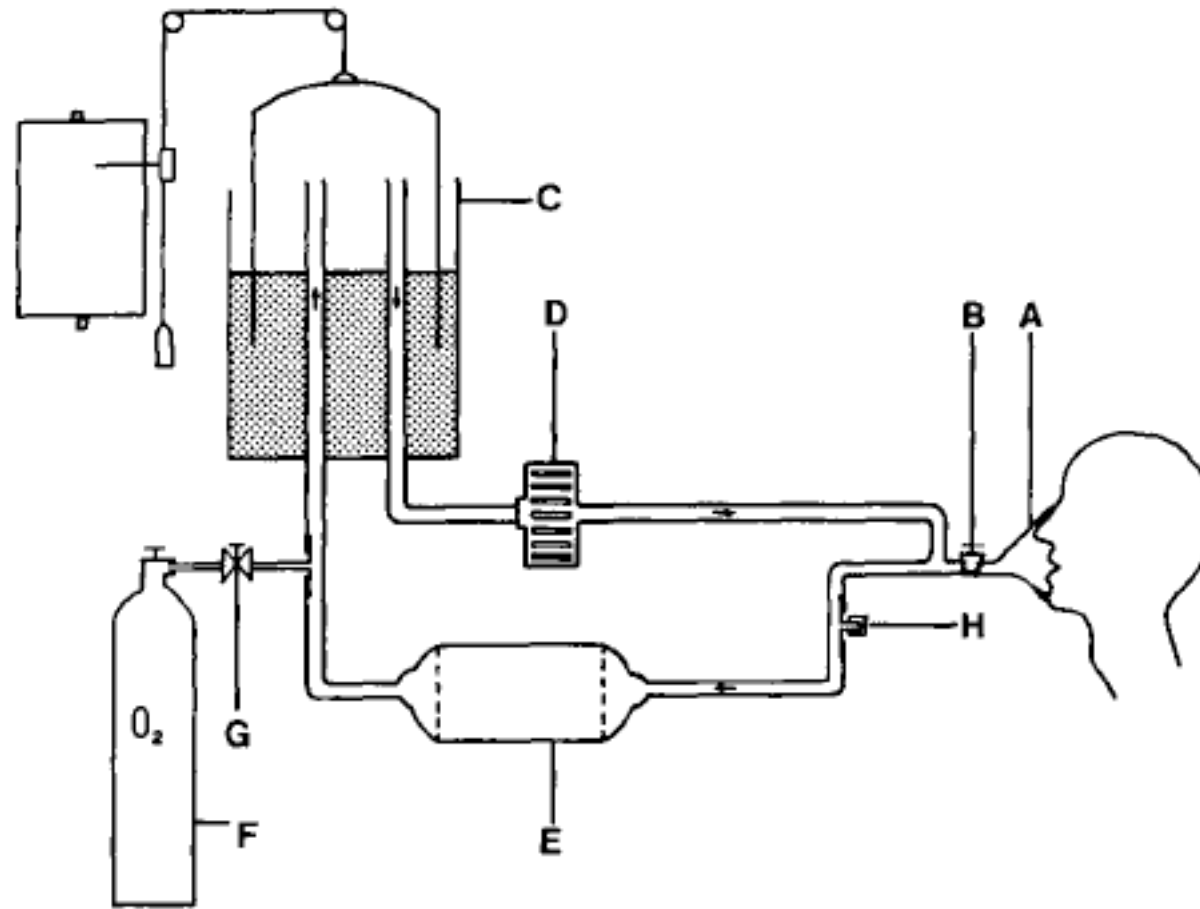
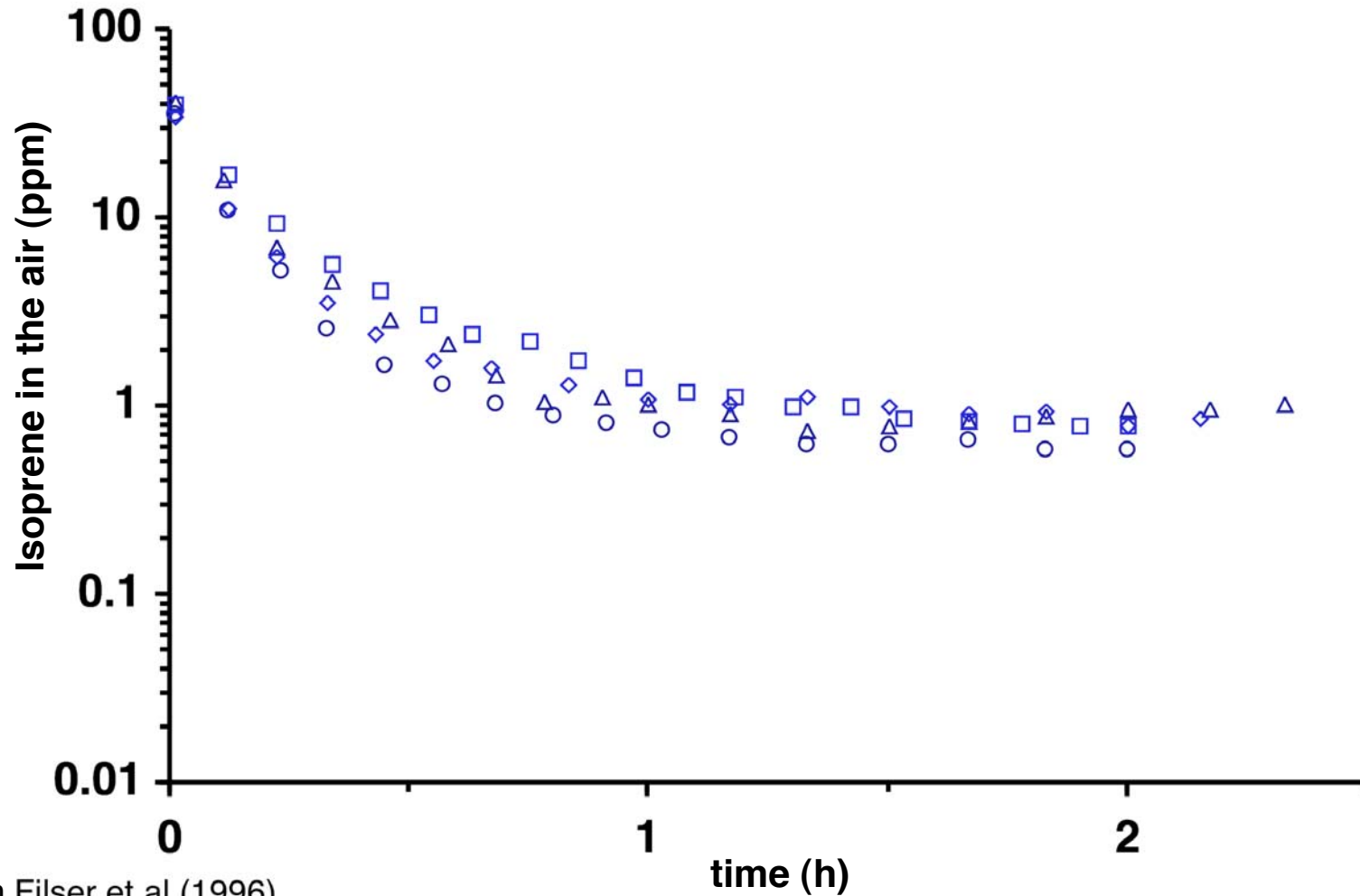


Fig. 2. Spirometer system for exposing humans to hydrophobic volatile substances. Symbols are specified in text

Inhalation experiments with isoprene II

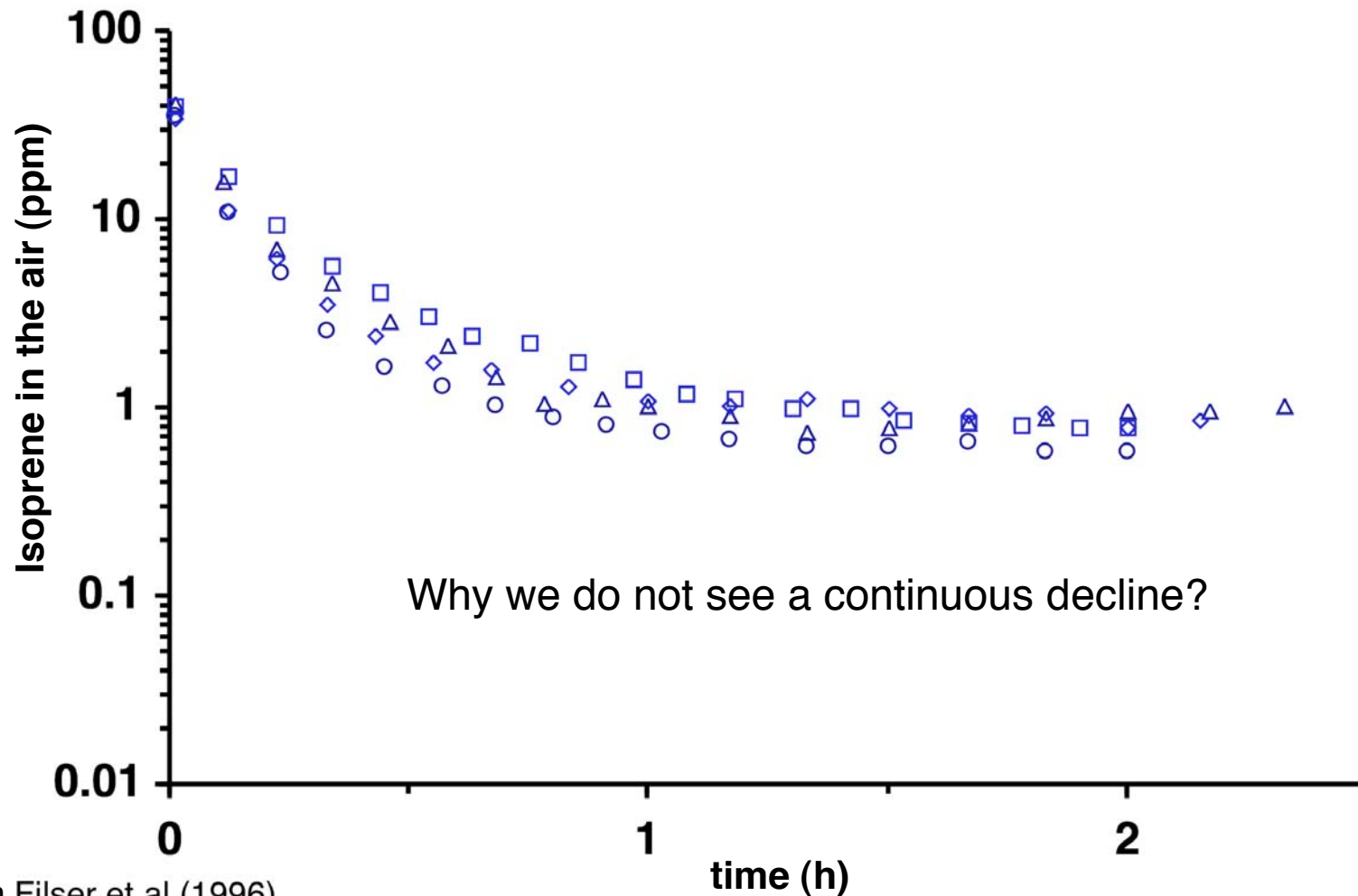
Concentration-time course of inhaled isoprene in the atmosphere of a spirometer system following exposure of volunteers to initial concentrations of about 40 ppm



Data from Filser et al (1996)

Inhalation experiments with isoprene III

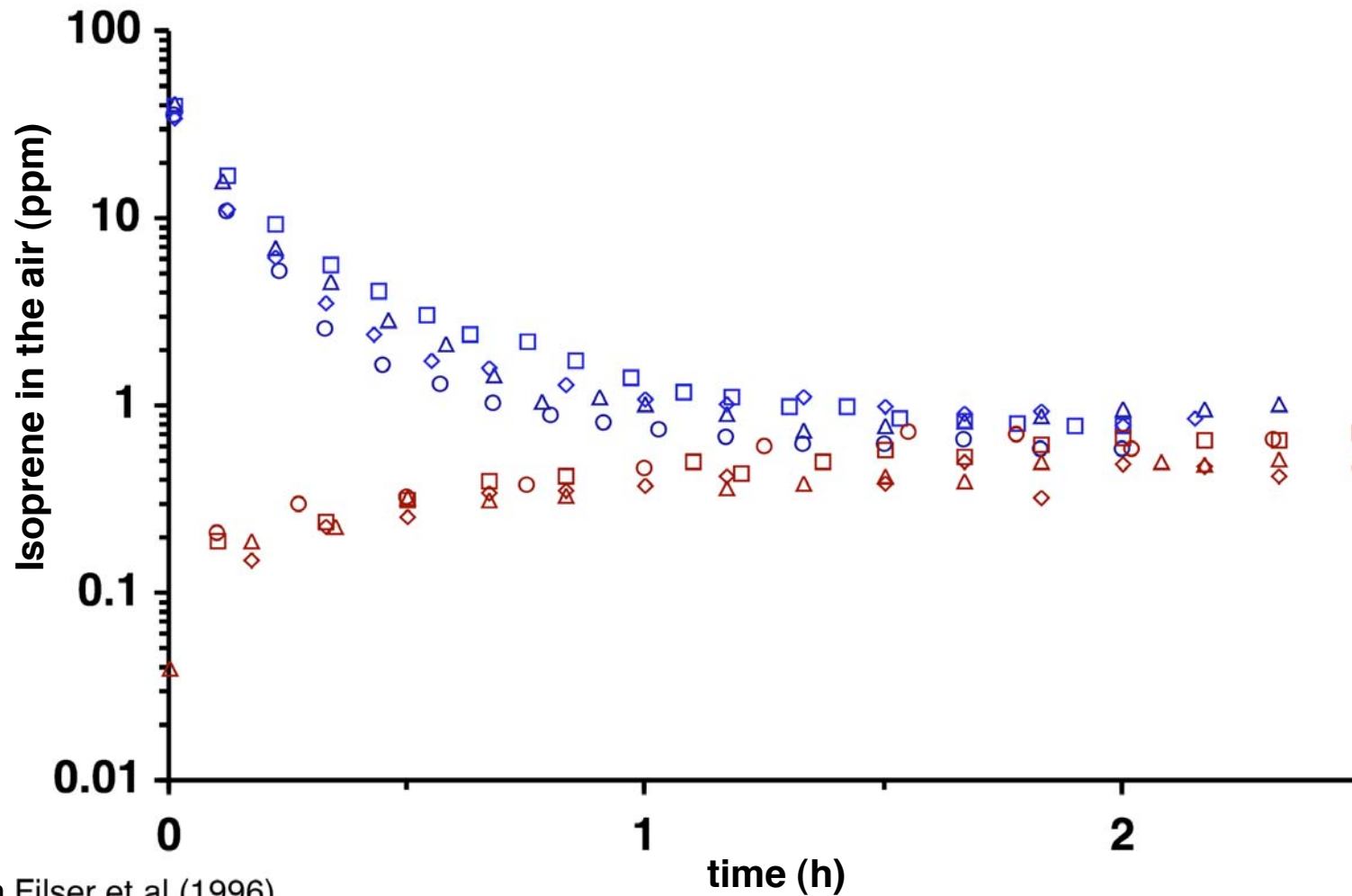
Concentration-time course of inhaled isoprene in the atmosphere of a spirometer system following exposure of volunteers to initial concentrations of about 40 ppm



Data from Filser et al (1996)

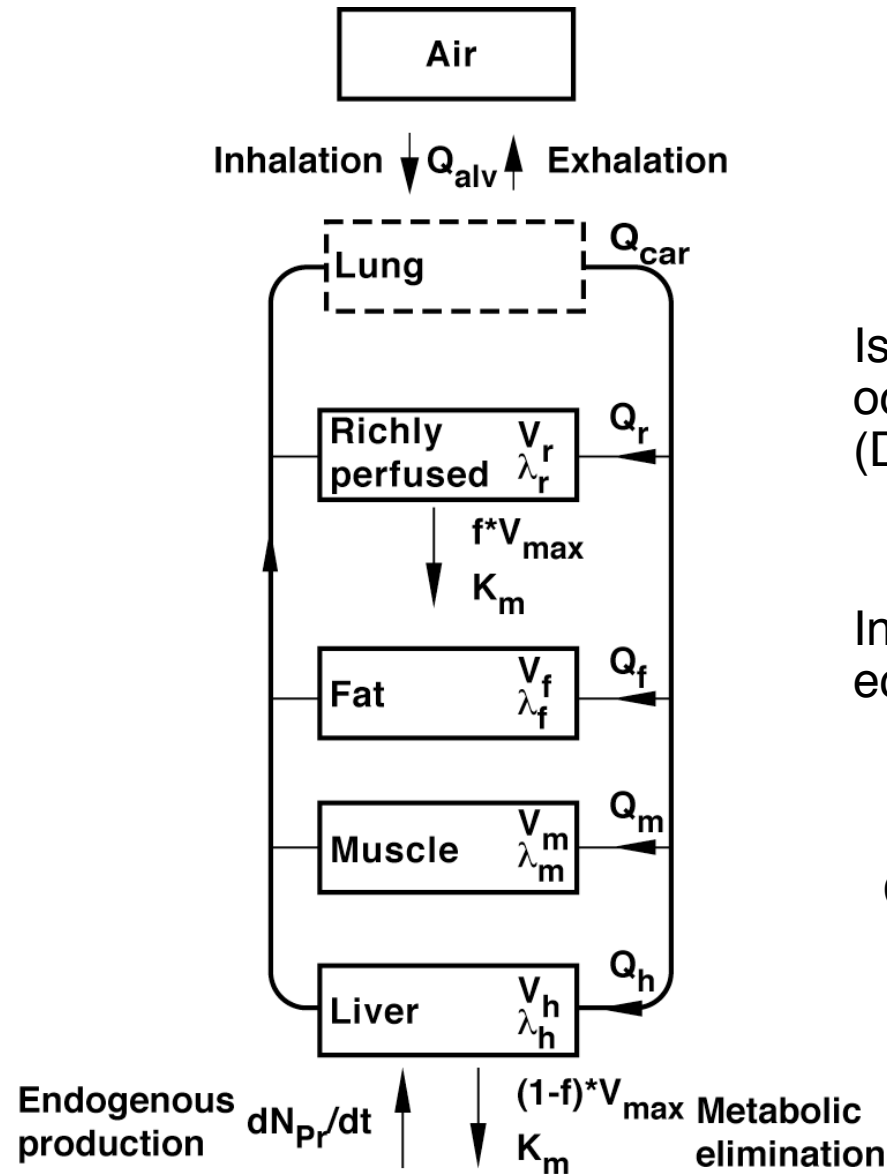
Inhalation experiments with isoprene III

Concentration-time curves of inhaled isoprene in the atmosphere of a spirometer system following exposure of volunteers to initial concentrations of 0 and 40 ppm



Data from Filser et al (1996)

Model improvement II



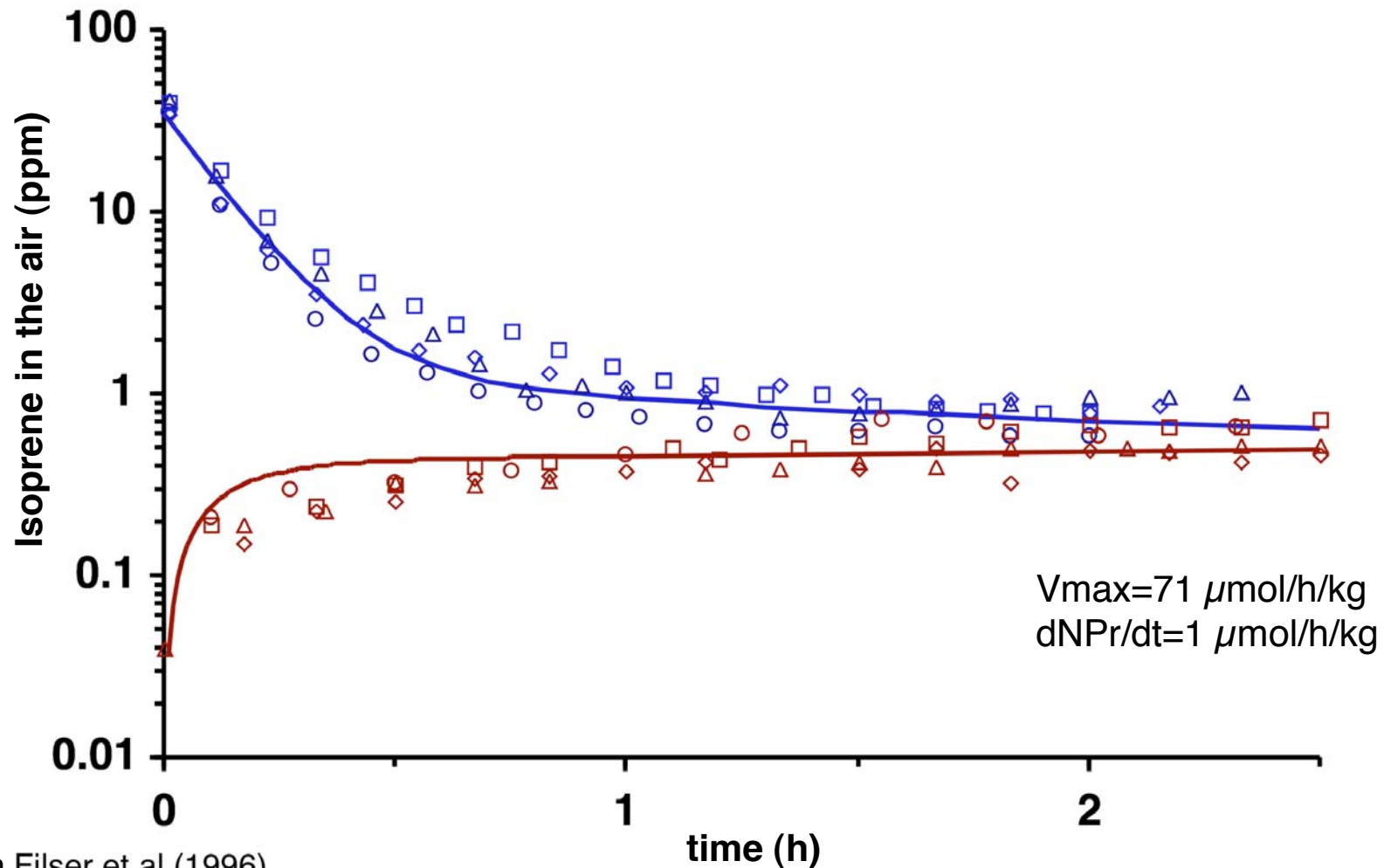
Isoprene production has been shown to occur in liver cytosol from mevalonic acid (Deneris et al. 1985).

Initial conditions for each differential equation must be calculated:

$$C_f(0) = \frac{\frac{dN_{Pr}}{dt} * \frac{1}{Q_{alv}}}{1 + \frac{\hat{k}_{el} * V_h}{Q_h} * \left(1 + \frac{Q_h * \lambda_{ba}}{Q_{alv}}\right)} * \lambda_f$$

Inhalation experiments with isoprene III

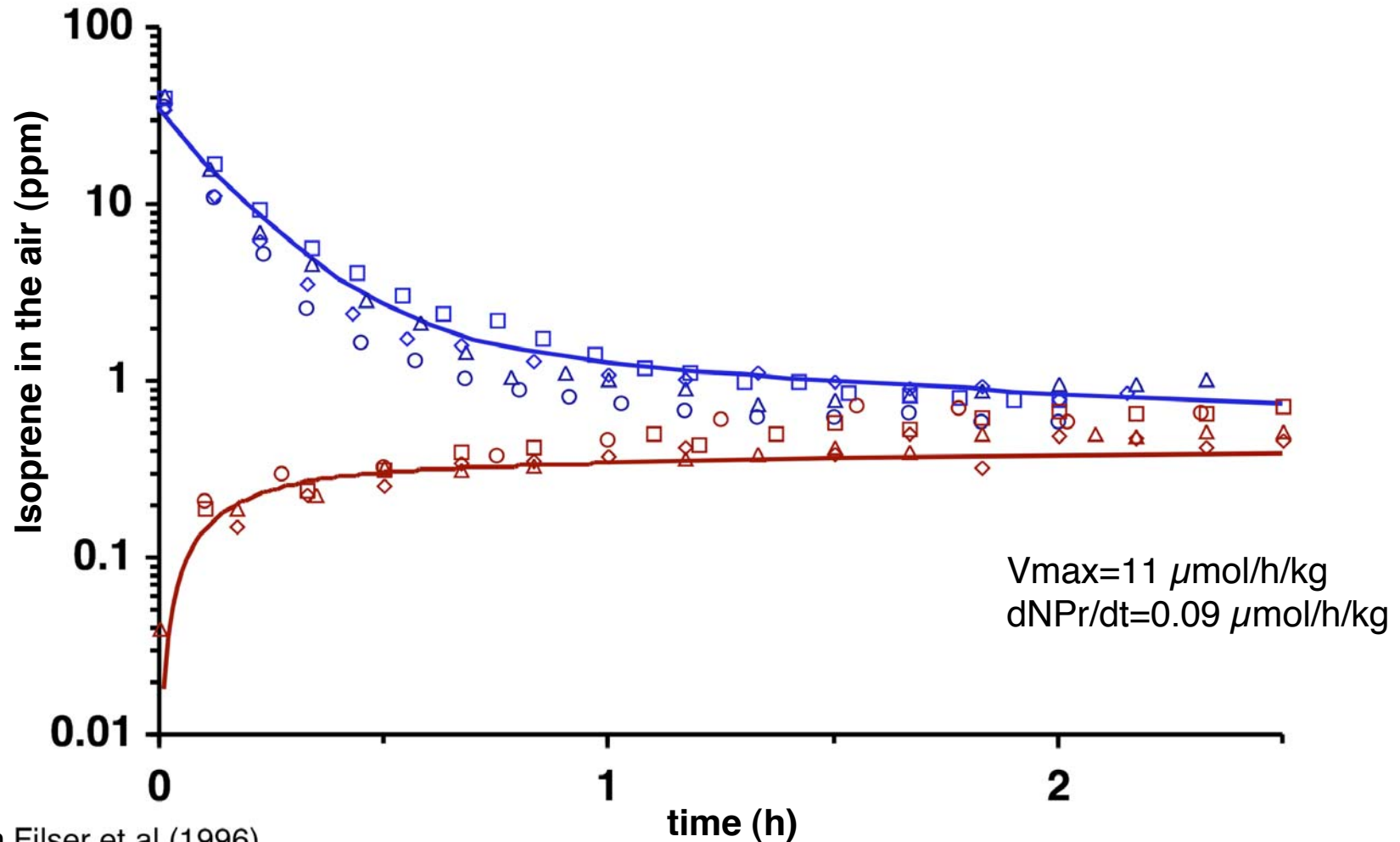
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Inhalation experiments with isoprene III

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Inhalation experiments with isoprene III

In order to determine the values of the metabolic parameters and the endogenous production rate independent data are required.

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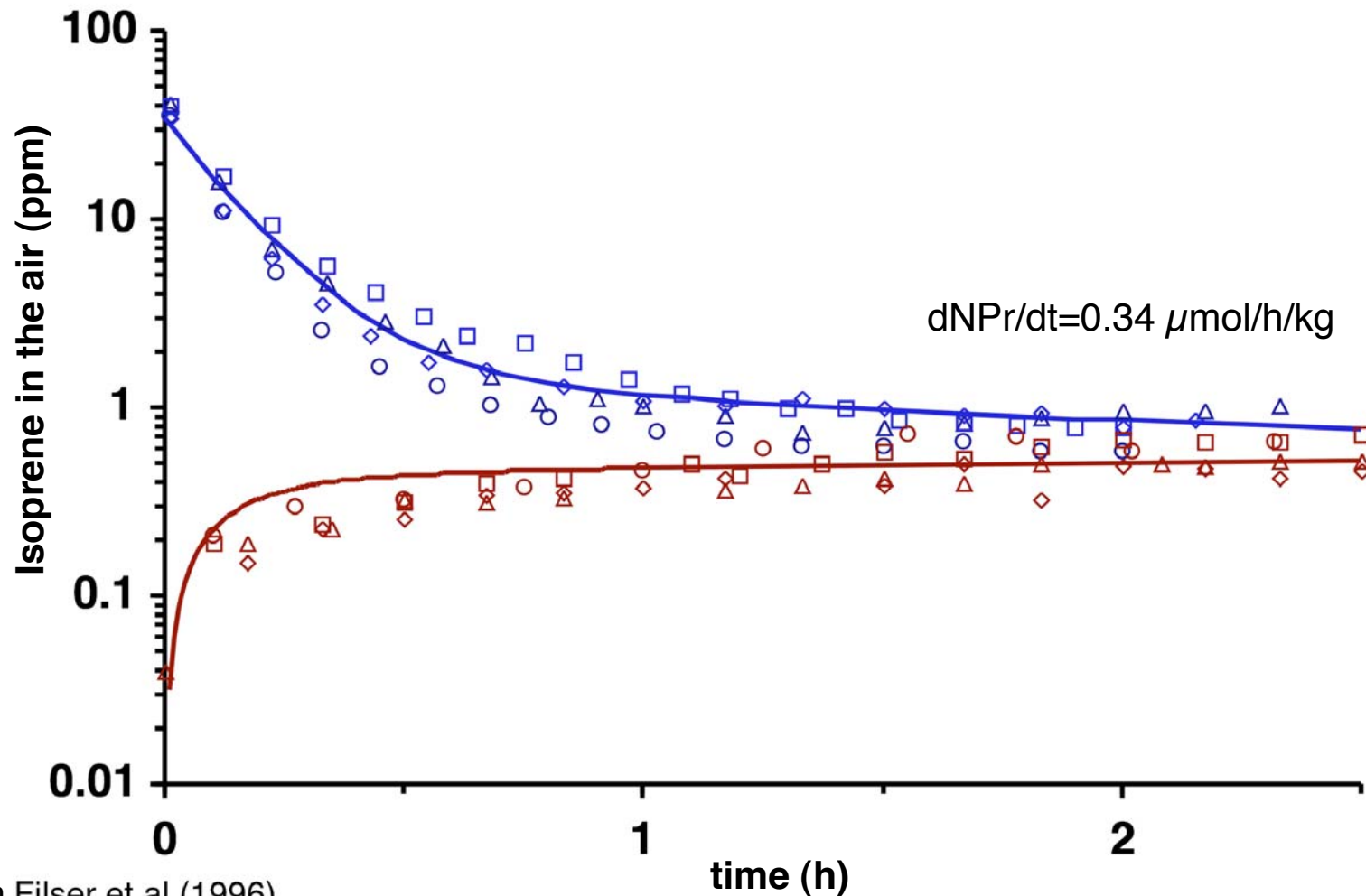
One possible approach is to use allometric scaling:

$$V_{\max}^{\text{Human}} = V_{\max}^{\text{Rat}} * (70 / 0.25)^{3/4} = 110 / 4 * (70 / 0.25)^{3/4} = 1880 \mu\text{mol} / \text{h}$$

The Km values are assumed to be identical.

Inhalation experiments with isoprene III

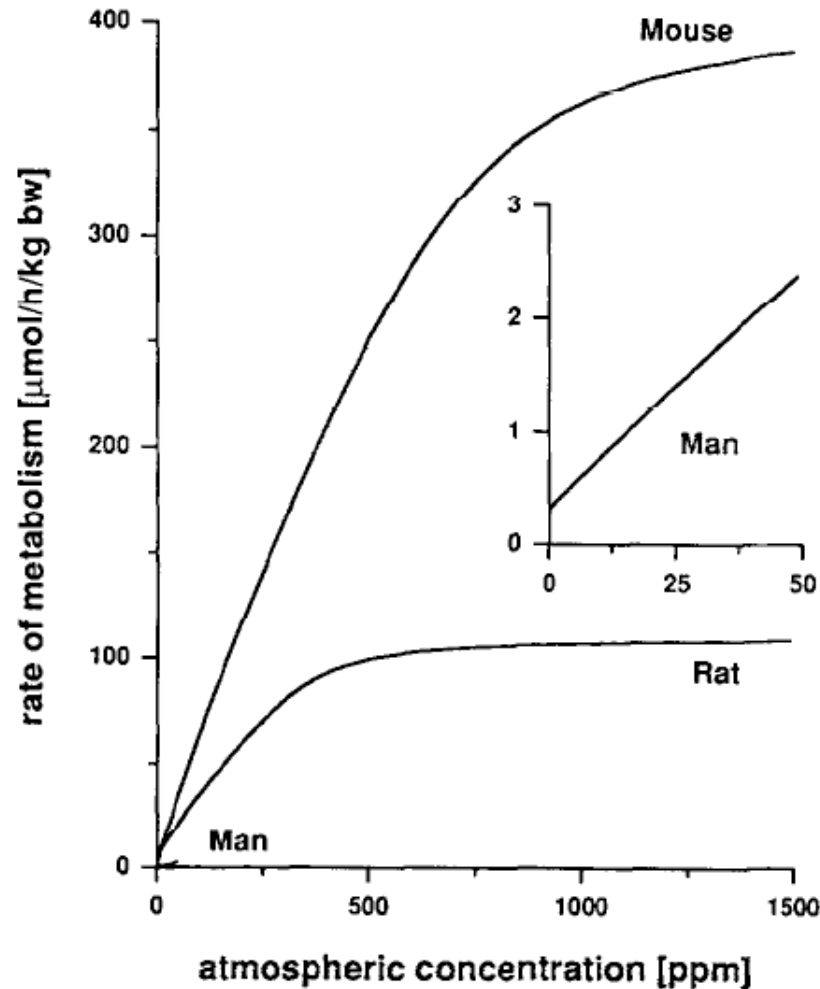
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Data from Filser et al (1996)

Outlook

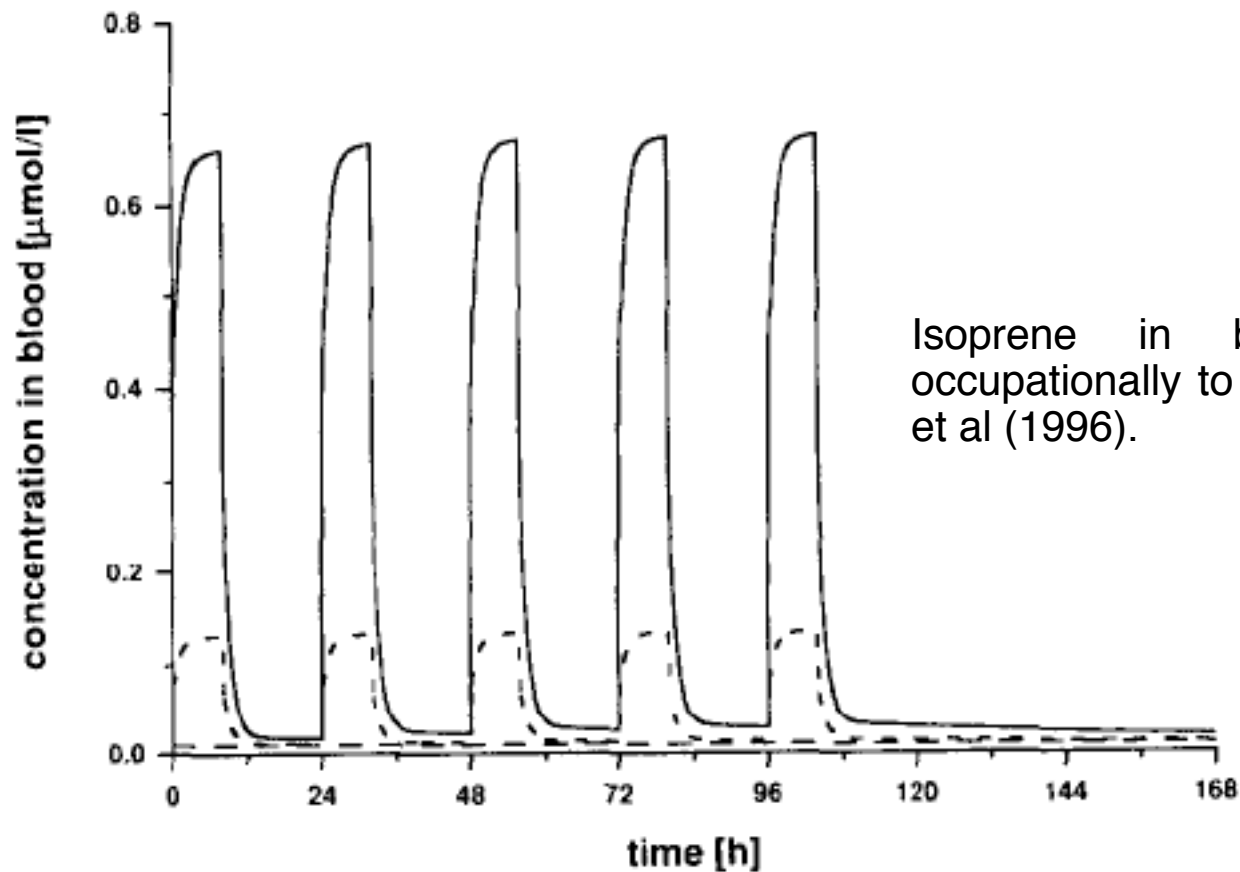
Once the physiological toxicokinetic model is established, it can be used to simulate interesting exposure scenarios:



Rate of isoprene metabolism in mouse, rat, and human at steady state in dependence of exposure concentration Filser et al (1996).

Outlook

Once the physiological toxicokinetic model is established, it can be used to simulate interesting exposure scenarios:



Isoprene in blood of human exposed occupationally to 10 and 50 ppm isoprene Filser et al (1996).

Summary

The development of physiological toxicokinetic models is a complex iterative process, requiring:

- data
- data, and
- data.
- expertise from the fields of mathematics, biochemistry, and toxicology.