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:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1.9-2.6 l</td>
</tr>
<tr>
<td>Food</td>
<td>1.4-1.7 kg</td>
</tr>
<tr>
<td>Air (inhaled)</td>
<td>21-24 m³ or 24-29 kg</td>
</tr>
</tbody>
</table>

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

\[ \text{Cart} = \frac{Q_{\text{car}} \sum Qi \cdot Cvi / \sum Qi + Q_{\text{alv}} \cdot C_{\text{exp}}}{Q_{\text{car}} + \frac{Q_{\text{alv}}}{\lambda_{\text{blood:air}}}} \]

\[ V_{\text{Cham}} \frac{dC_{\text{exp}}}{dt} = Q_{\text{alv}} \left( \frac{\text{Cart}}{\lambda_{\text{blood:air}}} - C_{\text{exp}} \right), \quad C_{\text{exp}}(0) = C_0 \]

\[ V_r \frac{dC_r}{dt} = Q_r \left( \text{Cart} - \frac{C_r}{\lambda_r} \right), \quad C_r(0) = 0 \]

\[ V_f \frac{dC_f}{dt} = Q_f \left( \text{Cart} - \frac{C_f}{\lambda_f} \right), \quad C_f(0) = 0 \]

\[ V_m \frac{dC_m}{dt} = Q_m \left( \text{Cart} - \frac{C_m}{\lambda_m} \right), \quad C_m(0) = 0 \]

\[ V_h \frac{dC_h}{dt} = Q_h \left( \text{Cart} - \frac{C_h}{\lambda_h} \right) - \frac{V_{\text{max}} \cdot C_h}{K_m + C_h}, \quad C_h(0) = 0 \]
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

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

“Reference values” form Arms and Travis (1988)

* reduced to 60% of the reference value
## Partition coefficients of isoprene

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

<table>
<thead>
<tr>
<th>Partition coefficient</th>
<th>Mouse</th>
<th>Rat</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood:air</td>
<td>2.04</td>
<td>2.33</td>
<td>0.75</td>
</tr>
<tr>
<td>Fat:blood</td>
<td>30.2</td>
<td>26.4</td>
<td>82</td>
</tr>
<tr>
<td>Muscle:blood</td>
<td>0.73</td>
<td>0.64</td>
<td>1.97</td>
</tr>
<tr>
<td>Liver:blood</td>
<td>0.95</td>
<td>0.83</td>
<td>2.57</td>
</tr>
<tr>
<td>Richly perfused tissues:blood*</td>
<td>0.91</td>
<td>0.79</td>
<td>2.45</td>
</tr>
</tbody>
</table>

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

Data from Peter et al (1987)

Inhalation experiments with isoprene I
Inhalation experiments with isoprene I

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

Data from Peter et al (1987)

Vmax=6.5 μmol/h/kg
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)
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).

Fig. 1. Concentration-time curves of isoprene in clo by 2 rats and 5 mice, respectively, in each exper
graphical extrapolation.
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

Filser et al (1992)

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<tr>
<th>Parameter</th>
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<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$ ($\mu$mol/h/kg)</td>
<td>410</td>
<td>110</td>
</tr>
<tr>
<td>$K_{\text{m}}$ (mmol/l)</td>
<td>0.06</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Parameters from Filser et al (1996)
First toxicokinetic analysis of isoprene data

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

<table>
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<th>Mouse (μmol/h/kg)</th>
<th>Rat (μmol/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax</td>
<td>410</td>
<td>110</td>
</tr>
<tr>
<td>Km</td>
<td>0.06</td>
<td>0.026</td>
</tr>
<tr>
<td>KmPT</td>
<td>0.004</td>
<td>0.002</td>
</tr>
</tbody>
</table>

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)
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)

Vmax=410 $\mu$mol/h/kg
Km=0.004 mmol/l
Inhalation experiments with isoprene II

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

\[ V_{\text{max}} = 410 \, \mu\text{mol/h/kg} \]
\[ K_m = 0.004 \, \text{mmol/l} \]

Data from Peter et al (1987)
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)

Vmax=1000 μmol/h/kg
Km=0.004 mmol/l
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

The liver clearance:

\[ Cl_H = \frac{C_{in} - C}{C_{in}} \times Q_h = \frac{Q_h \times Cl_{int}}{Q_h + Cl_{int}} \]

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

Wilkinson and Shand (1975)

If \( \lim_{Cl_{int} \to \infty} Cl_H \to Q_h \)
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

<table>
<thead>
<tr>
<th>time (h)</th>
<th>Isoprene in the air (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>≤100</td>
</tr>
<tr>
<td>1</td>
<td>≤100</td>
</tr>
<tr>
<td>2</td>
<td>≤100</td>
</tr>
<tr>
<td>4</td>
<td>≤100</td>
</tr>
<tr>
<td>6</td>
<td>≤100</td>
</tr>
<tr>
<td>8</td>
<td>≤100</td>
</tr>
<tr>
<td>10</td>
<td>≤100</td>
</tr>
</tbody>
</table>

Data from Peter et al (1987)

\[ V_{\text{max}} = 410 \, \mu\text{mol/h/kg} \]

\[ K_{\text{m}} = 0.004 \, \text{mmol/l} \]
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 diethylthiocarbamate pretreated (△) and in naïve animals (○). 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 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 course of inhaled isoprene in the atmosphere of a spirometer system following exposure of volunteers to initial concentrations of about 40 ppm

Why we do not see a continuous decline?

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)
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:

$$C_f(0) = \frac{dN_{Pr} \cdot 1}{dt \cdot Q_{alv}} \cdot \frac{\lambda_f}{1 + \frac{k_{el} \cdot V_h}{Q_h} \cdot (1 + \frac{Q_{lh} \cdot \lambda_{ba}}{Q_{alv}})}$$
Inhalation experiments with isoprene III

Concentration-time curses 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)

Vmax=71 μmol/h/kg
dNPr/dt=1 μmol/h/kg
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

\[ V_{\text{max}} = 11 \, \mu\text{mol/h/kg} \]
\[ \frac{dNPr}{dt} = 0.09 \, \mu\text{mol/h/kg} \]

Data from Filser et al (1996)

Institute of Toxicology
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:

\[ V_{\text{max}}^{\text{Human}} = V_{\text{max}}^{\text{Rat}} \times (70 / 0.25)^{3/4} = 110 / 4 \times (70 / 0.25)^{3/4} = 1880 \, \mu\text{mol} / \text{h} \]

The Km values are assumed to be identical.
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)

dNPr/dt=0.34 µmol/h/kg
Once the physiological toxicokinetic model is established, it can be used to simulate interesting exposure scenarios:

Outlook

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

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.