Pharmacokinetics and Metabolism (Toxicokinetics)

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Preclinical Disciplines

• **Pharmacology**
  – Research on desirable effects by in vitro and in vivo experiments

• **Pharmaco-Toxicokinetics examines**
  – Systemic and local exposure,
    e.g. AUC = Area Under the Curve, \( c_{\text{max}} \), \( t_{\text{max}} \)

• **Toxicology analysis**
  – adverse effects by in vitro and in vivo models as prediction for human use,
    kinetic data always incorporated.
Functions of preclinical Disciplines

- **Pharmacology und Toxicology**: what does the drug do with the body? desirable and undesirable effects.

- **Kinetic (Pharmacokinetics)**: what does the body do with the drug?
Pharmacokinetics

1. Investigates, which influence the body exercises on the drug.

2. **What does the body with the drug?**
   - A = Absorption
   - D = Distribution
   - M = Metabolism
   - E = Excretion

3. Objectives:
   - dose-effect relationship,
   - optimal dose level determination.
ADME is part of the Pharmakokinetik:

Absorption

Distribution

Metabolismus

Excretion
Objectives of Kinetics:

How does the drug get into the body?
Where is the drug distributed in the body?
How much of the administered drug is available for any effects?
Parent compound? Prodrug? Metabolites?
Kinetics at receptor?
Excretion via urine, gall bladder, respiration?
Half life time t_{1/2}?
Maximum concentration? c_{max}? t_{max}?
Pharmacokinetic Processes:

- Intake
  - Dissolution
    - Absorption
      - Distribution
        - Accumulation
      - Metabolism
        - Excretion

Pharmacodynamic Effects:

- Therapeutic
- Toxic
Toxicokinetic Monitoring or Profiling

- **Monitoring** = 1 – 3 samples during dosing interval
  - to estimate $C_{\text{max}}$ or $C_{(\text{time})}$
  - timing: start + end of study

- **Profiling** = 4 – 8 samples
  - to estimate AUC, $C_{\text{max}}$ or $C_{(\text{time})}$
  - steady-state concentrations, 1 or more time points
Kinetics = Tool for interspecies Extrapolation

• Extrapolation from animal to humans: this only optimal, when animal model is „the most human like species“:
  – comparable pharmacodynamics of parent compound and metabolites
  – comparable kinetic data as AUC, $C_{\text{max}} + t_{\text{max}}$, bioavailability, elimination, protein binding etc.
  – comparable adverse effect profile
Pharmaco- vs. Toxicokinetics

1. **Pharmacokinetics:**
   all data on chemical changes of the actual substance and its metabolites like
   - ADME,
   - oral bioavailability,
   - plasma half life
   - clearance
   - volume of distribution
   - mean residence time
   - protein binding: unbound and free fraction
   - steady state conditions etc.

2. **Toxicokinetics**
   all kinetic data from non-clinical safety studies, focus on AUC, $c_{\text{max}}$ and $t_{\text{max}}$
Toxicokinetics
Definition

• Toxicokinetics is defined as the generation of pharmacokinetic data, either as an integral component in the conduct of non-clinical toxicity studies or in specially designed supportive studies, in order to assess systemic exposure.
<table>
<thead>
<tr>
<th>Toxicokinetics</th>
<th>Pharmacokinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>- high doses</td>
<td>- low (pharmacological) doses</td>
</tr>
<tr>
<td>- integrated into toxicological studies or as supportive studies</td>
<td>- independent animal studies</td>
</tr>
<tr>
<td>- focussed on systemic exposure</td>
<td>- characterizing the basic pharmacokinetic properties</td>
</tr>
<tr>
<td>- reduced set of parameters (cmax, AUC, protein binding)</td>
<td>- complete set of parameters (Cl, volume of distribution, t_1/2, bioavailability)</td>
</tr>
<tr>
<td>- essential for interpretation / extrapolation of toxicological effects</td>
<td>- essential for description / extrapolation of pharmacokinetic behaviour</td>
</tr>
</tbody>
</table>
Regulatory Background

• ICH Guideline No. S3A (CPMP/ICH/384/95):

**Toxicokinetics:**
The assessment of systemic exposure in toxicity studies.
General Principles

• Toxicokinetic data must be performed according to Good Laboratory Practice (GLP).

• The exposure should be described by Cmax (peak concentration) and AUC (area under the curve) of parent compound and/or metabolites in the blood.

• In some circumstances, studies may be designed to investigate tissue concentrations.

➤ Example: concentration of fluoroquinolones in the eye due to the binding to melanin in the retina
General Principles

• Toxicokinetic investigations should be performed in an appropriate number of animals and dose groups.

• Sample collection from
  ➔ main study animals
    (in non-rodents frequent blood sampling is possible)
  ➔ satellite groups (rodents)
General Principles

• Contribution to the setting of dose levels in order to produce adequate exposure

⇒ **Low dose level**: should be the no-toxic-effect dose; the exposure should ideally be at least equal or better exceed the maximum expected exposure in patients.

⇒ **Intermediate dose level**: the exposure should represent an appropriate multiple of the exposure at the lower dose.

⇒ **High dose level**: will normally be determined by toxicological considerations (e.g. maximum tolerated dose).
General Principles

- **Determination of metabolites**

  ➔ when the administered drug acts as a pro-drug and the delivered metabolite is acknowledged to be the primary active entity

  ➔ when the compound is metabolised to one or more pharmacologically or toxicologically active metabolites

  ➔ when the administered compound is very extensively metabolised and measurement of plasma or tissue concentrations of a major metabolite is the only practical means of estimating exposure following administration of the compound in toxicity studies
Why do Kinetics matter in Toxicology?

Because:

• Species differences
  >> selection of appropriate dose level / dose interval

• Correlates toxicity with exposure

• Calculation of safety margins

>> Integrate ADME & Tox Data: ADMET!
In which toxicological studies should toxicokinetic investigations be performed?

- Single-Dose toxicity studies: usually not

- Repeat-dose toxicity studies: always

- Genotoxicity studies: may be helpful for the interpretation of *in vivo* studies

- Carcinogenicity studies: always (can be pivotal for the selection of the doses, especially the high dose)

- Reproductive toxicity studies: may be helpful
Is it $c_{\text{max}}$ or is it AUC which is inducing side effects/toxicity?

- **Functional effects**
  (e.g. CNS side or cardiovascular side effects) are often triggered by peak concentrations ($c_{\text{max}}$)

- **Morphological effects**
  (organ lesions) are often triggered by AUC
Toxicokinetics - Regulatory Background


2. THE OBJECTIVES OF TOXICOKINETICS AND THE PARAMETERS WHICH MAY BE DETERMINED

The primary objective of toxicokinetics is:

- to describe the systemic exposure achieved in animals and its relationship to dose level and the time course of the toxicity study.

Secondary objectives are:

- to relate the exposure achieved in toxicity studies to toxicological findings and contribute to the assessment of the relevance of these findings to clinical safety.

- to support (Note 1) the choice of species and treatment regimen in non-clinical toxicity studies.

- to provide information which, in conjunction with the toxicity findings, contributes to the design of subsequent non-clinical toxicity studies.
Toxicokinetics - Regulatory Background (Examples)

- EMEA CPMP/SWP/1042/99 corr., 27 July 2000: Note for Guidance on Repeated Dose Toxicity
  (also in new Draft EMEA/CHMP/SWP/488313/2007, 21 February 2008)

6.3 Toxicokinetics

Information on systemic exposure of animals during repeated dose toxicity studies are essential for the interpretation of study results, to the design of subsequent studies and to the human safety assessment. For detailed guidance see CPMP/ICH/384/95 (Note for guidance on Toxicokinetics: A Guidance for assessing systemic exposure in toxicology studies).
AUC = Area Under the Curve

i.v. administration
AUC = Area Under the Curve

Cmax, tmax

Oral administration

Time

Concentration
Absorption into the body

- Absorption describes the rate at which a drug leaves its site of administration and the extent to which this occurs.
Factors that modify Absorption

• Solubility of the drug:
  the more soluble the better the absorption

• Concentration of the drug in the solution:
  drugs in solutions of high concentration are absorbed more rapidly than are drugs in solutions of low concentration

• Circulation at the site of absorption:
  increased blood flow enhances the rate of drug absorption

• Area of the absorbing surface:
  large surface areas facilitate the absorption
  (pulmonary alveolar epithelium, intestinal mucosa)
Forms of Resorption

- **Passive diffusion** = transport follows concentration

- **Facilitated diffusion** = carrier molecules support process (structure specific, saturable,)

- **Active transport**
  Transport against concentration

- **Phagozytose** =
Absorption - Mechanisms

- Simple diffusion (hydrophob)
- Filtration (hydrophil, small)
- Facilitated diffusion (carrier, not energy-dependent)
- Active transport (carrier, energy-dependent)
- Phagocytosis
Phagocytosis

Rasterelektronic: a macrophage digest an erythrocyte

http://fachberatung-biologie.de
Prediction of Human Intestinal Absorption of Drug Compounds from Molecular Structure

by: M.D. Wessel et al. (Pennsylvania State University)

• Literature research on 86 compounds
  – 22 compounds absorb at 100%
  – 47 with absorption values at 90% or higher
  – 71 (=83% of total data set) absorb at 50% or higher
  – 15 absorb below 50%
  – 6 absorb below 5%:
    • gentamicin, cromolyn, olsalazin,
    • ganciclovir, cefuroxime, doxorubicin
Distribution within the Body

Transport via blood stream and tissue perfusion

- **Blood:**
  
  ➔ drug can be bound to erythrocytes and plasma proteins (albumin)
  
  ➔ most relevant for the activity and/or toxicity of a drug is the unbound or free fraction
Distribution within the Body

Transport via blood stream and tissue perfusion

- Capillary bed:
  - diffusion into tissues/site of action, receptor
  - permeability increases with lipophilicity
  - polar compounds have a poor permeability, especially at blood brain barrier
Distribution within the Body

- **Measurement technique:**

  - quantitative analysis of radioactivity or unchanged drug in organs (whole body autoradiography)
Distribution studies

- Tissue distribution studies are essential in providing information on distribution and accumulation of the compound and/or metabolites in relation to potential sites of action.
- The information is useful for the designing of toxicology and pharmacology studies and for interpreting the results of these experiments.

(direction) Identification of possible target organs for toxicity
Distribution studies

• Usually **single-dose studies** provide sufficient information about tissue distribution.

• However, there may be circumstances when assessments after **repeated dosing** may yield important information.
Distribution studies are important to investigate

- if a drug can penetrate the **placental barrier** or can be secreted into the milk
- studies in pregnant animals (rats)
- information for the label:

  ➔ *Information on excretion of the active substance and/or its metabolite(s) in milk should be given*
  ➔ *Where relevant, recommendation as to whether to stop or continue breast feeding should be given*
Kinetics / Radioactivity

- Modern analytical methods can detect very small quantities of parent drug or metabolites.
- Relevant tools are radioactive labelling by 14C or 3H (Tritium).
- The labelled compounds are administered to animals, then distribution in body are examined.
Whole Body Autoradiography

- Detection of radiolabeling in biologic samples
- Autoradiograms reflect intensity and local distribution of radioactive atoms
- Radiolabeling usually with alpha- oder beta rays
- For drugs: usually rats are used
WBA in the rat

Study-no: R359/FKM/201
Dose: 5 mg/kg p.o.

t = 0.25 h
WBA in the rat p.o., 1 h

Study no: R359/FKM/201
Dose: 5 mg/kg p.o.

t - 1 h

intestine  stomach wall  liver  brain
WBA in the rat p.o., 8 h

Study no: R359/FKM/201
Dose: 5 mg/kg p.o.

faeces

skin

liver

stomach wall

t = 8 h
Distribution Areas of the Organism

Percentage of Body Weight

Plasma: 5%
Interstitial Space: 15%
Intracellular Space: 40%
Body Fluids: 3%
Inaccessible Water: 7%
Dry Masses: 15%
Body Fat: 15%
“Metabolism (biotransformation) is the sum of the processes by which a foreign chemical is subjected to chemical change by living organisms.“

(EPA OPPTS 870.7485 ‘Metabolism and Pharmacokinetics‘)
Why does Metabolism matter in Toxicology?

Because:

- Formation of (toxic) metabolites
  >> xenobiotics may be toxified or detoxified

- Species differences
  >> selection of appropriate tox species

>> Integrate ADME & Tox Data: ADMET!
Metabolism – In vitro Test Systems

- Recombinant enzymes (CYPs, phase II, e.g. in V79 cells, Supersomes®, ...)
- Permanent cell lines (HepG2, ...)
- Tissue (liver) homogenate (S9, cytosol)
- Tissue (liver) microsomes + cofactors (phase I, UGT, no phase II)
- Isolates hepatocytes (fresh, cryopreserved, suspension, plated)
- Tissue (liver) slices
- Tissue (liver) perfusion
- Transgenic mice (hepatic CYP reductase null “HRN” mouse)
- ...

…
Metabolism - Liver / Hepatocytes

Collagen Sandwich Culture of Rat Primary Hepatocytes

untreated control

30 µm

30 µm

treated with allyl alcohol
(10 µg/ml, 48 h)
CYPs - Quantitative Significance in human Drug Metabolism

- 2A6, 2B6, 2C8, 2C18, 2C19, 4A11, 4B1
- 2D6
- 2C9
- 1A2
- 1A1
- 1B1
- 3A4 (3A5, 3A7?)
- Extrahepatic forms?
CYPs - Reactions

- Aliphatic and aromatic hydroxylation
- Epoxidation
- N-, S- Oxidation
- N-, O-, S- Dealkylation
- Oxidative desamination
- Desulfuration
- Oxidative and reductive dehalogenation
- Dehydrogenation
- Reduction (e.g., of azo and aromatic nitro groups)
### CYPs – The ‘Common’ Hepatic Ones …

<table>
<thead>
<tr>
<th>CYP</th>
<th>Content [of total]</th>
<th>Comments</th>
<th>Substrate Characteristics: Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>13%</td>
<td>Inducible (omeprazole)</td>
<td>Aromatic and heterocyclic amines, PAH, ethoxyresorufin, caffeine</td>
</tr>
<tr>
<td>2A6</td>
<td>4%</td>
<td>Inducible (pyrazole)</td>
<td>Coumarine, diethylnitrosamine</td>
</tr>
<tr>
<td>2B6</td>
<td>1-15%</td>
<td>Polymorphic, inducible (PB, less than in rodents)</td>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td>2C8</td>
<td></td>
<td>Polymorphic, inducible (PB)</td>
<td>Paclitaxel</td>
</tr>
<tr>
<td>2C9</td>
<td></td>
<td>Polymorphic, inducible (PB)</td>
<td>Many: diclofenac</td>
</tr>
<tr>
<td>2C18</td>
<td>18%</td>
<td>Polymorphic, inducible (PB)</td>
<td>Methenytoin, omeprazole</td>
</tr>
<tr>
<td>2C19</td>
<td></td>
<td>Polymorphic, inducible (PB)</td>
<td>Many drugs, more hydrophilic with basic nitrogen: sparteine, debrisoquine, bufuralol</td>
</tr>
<tr>
<td>2D6</td>
<td>2.5%</td>
<td>Polymorphic, not inducible</td>
<td>Many drugs, more hydrophilic with basic nitrogen: sparteine, debrisoquine, bufuralol</td>
</tr>
<tr>
<td>2E1</td>
<td>7-15%</td>
<td>Lung, inducible (ethanol)</td>
<td>Small molecules: ethanol, acetaminophen, benzene, carbon tetrachloride, chloroform</td>
</tr>
<tr>
<td>3A4</td>
<td>30%</td>
<td>Intestine, inducible (rifampicin), most abundant in human liver</td>
<td>Many: aflatoxins, midazolam, testosterone</td>
</tr>
<tr>
<td>3A5</td>
<td>5%</td>
<td></td>
<td>Nifedipine</td>
</tr>
</tbody>
</table>
# CYPs - Marker Reactions & Reference Inhibitors

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Marker Reaction</th>
<th>Reference Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1/2</td>
<td>ethoxyresorufin O-deethylation</td>
<td>furafylline</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>coumarin 7-hydroxylation</td>
<td>8-methoxypsoralene</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>bupropion hydroxylation</td>
<td>triethylenetriothiophosphoramide</td>
</tr>
<tr>
<td></td>
<td>S-mephenytoin N-demethylation</td>
<td></td>
</tr>
<tr>
<td>CYP2C8</td>
<td>paclitaxel 6α-hydroxylation</td>
<td>ketoconazole</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>tolbutamide 4-hydroxylation</td>
<td>sulfaphenazole</td>
</tr>
<tr>
<td></td>
<td>diclofenac 4'-hydroxylation</td>
<td></td>
</tr>
<tr>
<td>CYP2C19</td>
<td>S-mephenytoin 4-hydroxylation</td>
<td>omeprazole</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>dextromethorphan O-demethylation</td>
<td>quinidine</td>
</tr>
<tr>
<td></td>
<td>bufuralol -hydroxylation</td>
<td></td>
</tr>
<tr>
<td>CYP2E1</td>
<td>chlorzoxazone 6-hydroxylation</td>
<td>diethyldithiocarbamate</td>
</tr>
<tr>
<td>CYP3A4/5</td>
<td>testosterone 6β-hydroxylation</td>
<td>ketoconazole</td>
</tr>
</tbody>
</table>

Non-selective inhibitors to block CYP activity: piperonyl butoxide, SKF 525A, CO
Metabolism: Species Differences

- **Dog**: low acetylation, high capacity for deacetylation, different absorption due to higher pH in GI than in humans (consider use synthetic gastric fluid to mimic human situation)
- **Rat**: often gender differences which are not observed in other species, abundant tetrahydrofolate protects against methanol ocular damage, less formation of NAPQI protects against acetaminophen liver damage
- **Rabbit**: low sulfation
- **Pig**: low sulfation, GI conditions similar to humans
- **Cat**: low glucuronidation, high sulfation

- Rate of metabolism: mouse > rat > dog > human
Metabolism
(Enzymatic Transformation)

Phase I-Reactions:

• oxidative hydroxylations, demethylations, hydrolysis etc.

• Cytochrome P450: heme containing enzyme system in the endoplasmatic reticulum of the liver and the small intestines

⇒ Genetically determined families of enzymes, e.g. CYP 3A4, CYP 2D6

⇒ Important for drug interactions
CYP 3A4

- the most important Cytochrome P 450 subtype

- located in the gut wall

- located in the liver

- 50% of drugs metabolised via the cytochrome P 450 system are substrates for CYP 3A4

- drugs may inhibit or induce CYP 3A4, i.e. the activity of the enzyme is reduced or increased
Inhibition of CYP 3A4

Consequences:

• reduced metabolism

• reduced elimination

• increased drug concentrations

• increased toxicity (important for drugs which have a narrow therapeutic window)
Inhibition of CYP 3A4

Examples:

• increased risk of arrhythmias with terfenadine when given in combination with drugs that inhibit CYP 3A4

• increased risk of rhabdomyolysis for some statins when given in combination with drugs that inhibit CYP 3A4
Induction of CYP 3A4

Consequences:

• increased metabolization of other drugs or its own metabolisation (autoinduction)

• increased elimination

• reduced drug concentrations

• loss of efficacy
Induction of CYP 3A4

Examples:

• undesired pregnancy due to a loss of efficacy of oral contraceptives, when given in combination with enzyme-inducing drugs
4-w-tox in the rat, 14 mg/kg p.o., Day 1

HPLC, fluorescence detection

BYK280958

3-hydroxymethyl metabolite

hydroxyethoxy metabolite
Hydroxyethoxy, 3-hydroxymethyl metabolite

Further polar metabolites

BYK280958

HPLC, fluorescence detection

No glucuronide
HPLC, fluorescence detection

kinetics in man, subject 1

BYK280958

20 mg, Day 1 / 7

10 mg, Day 1 / 7

glucuronide

3-hydroxymethyl metabolite

hydroxyethoxy-metaboltite

50.0

100.0

150.0

200.0

250.0

300.0

350.0

400.0

450.0

500.0

550.0

600.0

650.0

700.0

750.0

800.0

[min]

[m AU]
Plasma Half Life (t $\frac{1}{2}$)

- Elimination kinetics 1. order; elimination is dependant on plasma concentration.

- $t \frac{1}{2}$ = time in which plasma concentration is decreased by 50%

- $t \frac{1}{2}$ = „hybrid“ parameter, depends on distribution and not on elimination

- Half life time $t \frac{1}{2}$: the longer, the bigger distribution volume; the shorter, the higher clearance
## Single Dose Pharmacokinetic Data for Drug X

<table>
<thead>
<tr>
<th>Species</th>
<th>Oral dose (mg/kg)</th>
<th>Cmax (µg/ml)</th>
<th>AUC (mg.h/l)</th>
<th>T ½ (H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>22</td>
<td>10</td>
<td>160</td>
<td>1</td>
</tr>
<tr>
<td>Mouse</td>
<td>32</td>
<td>24</td>
<td>413</td>
<td>3</td>
</tr>
<tr>
<td>Dog</td>
<td>2</td>
<td>9</td>
<td>400</td>
<td>46</td>
</tr>
<tr>
<td>Man</td>
<td>0.8</td>
<td>11</td>
<td>630</td>
<td>60</td>
</tr>
</tbody>
</table>
Metabolismus
First-pass-Effekt

- Metabolism at location of absorption
- e.g. enteral absorption
Excretion

Bile excretion and enterohepatic circulation

- Phase II-Metabolites („conjugates“) = most molecules, are preferably excreted via bile
- Glukuronides can be regained in the intestinal lumen by bacterial glucoronidases
- Parent compound and also metabolites (phase I) can be re-absorbed in the intestines
Excretion

Routes:

- biliary/fecal, renal, cutaneous, pulmonary

- liver and renal clearance limit the maximal excretion
Bioavailability

- is a measure for the proportion of the dose that reaches the systemic circulation
- is often the limiting factor in case of missing or poor activity after oral administration
- may be the reason for underestimating toxicity, if the bioavailability of a drug is high in humans and low in the animal species used in the toxicological studies
“Bioavailability“

depends on several factors such as

• stability in the gastro-intestinal tract (pH, enzymes)

• permeation through the intestinal mucosa

• metabolic extraction during first pass (gut wall, liver)
Kinetics - Single Dose - Bioavailability

\[ C_{\text{max}} \text{ [}\mu\text{g/mL}\text{]} = \text{maximum plasma concentration} \]

\[ t_{\text{max}} \text{ [h]} = \text{time to } C_{\text{max}} \]

\[ \text{AUC [h}^*\mu\text{g/mL]} = \text{area under the curve} \]

\[ \text{Bioavailability [%]} = \frac{\text{AUC}_{\text{po}}}{\text{AUC}_{\text{iv}}} \]

\[ t_{1/2} \text{ [h]} = \text{terminal half-life} \]
Example:

Bioavailability of Moxifloxacin in Rat, Monkey, Dog and Man After Single Oral Administration

<table>
<thead>
<tr>
<th>Species</th>
<th>Rat</th>
<th>Monkey</th>
<th>Dog</th>
<th>Man</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailability [%]</td>
<td>78</td>
<td>52</td>
<td>91</td>
<td>91</td>
</tr>
</tbody>
</table>
„Clearance“

- is a measure for the rate of drug elimination
- can consist of
  - metabolic clearance (hepatic, non-hepatic)
  - renal (filtration, active transport)
  - others (e.g. biliary secretion)
„Clearance“

• determines exposure and half-life

⇒ high clearance - low exposure

⇒ high clearance - short half-life
„Clearance“

• is important for the duration of action and thus the dosing regimen (e.g. o.d. or b.i.d)

• is mostly dependent on elimination

• is species dependent and often longer in humans than in small rodents (can be a problem for the exposure which can be achieved in the toxicological studies)
Example:

Half-life of Moxifloxacin in Rat, Monkey, Dog and Man After Single Oral Administration

<table>
<thead>
<tr>
<th>Species</th>
<th>Rat</th>
<th>Monkey</th>
<th>Dog</th>
<th>Man</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; [h]</td>
<td>1.3</td>
<td>7.2</td>
<td>9.0</td>
<td>12</td>
</tr>
</tbody>
</table>
„Unbound Fraction“

- is the fraction of drug not bound to plasma proteins
- can have values down to less than 1% in plasma
- can be different between species (up to more than 10 fold)
- must be taken into account when calculating safety factors
Calculation of Safety factors
The Paracelsian approach:

• „All things are poison, for there is no thing without poisonous quality. It is only the **dose** which makes a thing a poison.“
  Paracelsus 1493 - 1541

The toxicokinetic approach:

• „It is only the **concentration** which makes a thing a poison.“
  Heykants 1993
Calculation of Safety factors

- **Historical approach:** Comparison of non-toxic doses in toxicological studies with highest therapeutic dose in man

- **New approach:** Comparison of non-toxic plasma concentrations in toxicological studies with highest therapeutic plasma concentration in man

- Margin of Safety or Therapeutic Window
Exposure =

- is a measure for the amount of drug that an organism has really „seen“ („local or systemic burden“)

- is dependent on dose, route, formulation, species

- allows a more meaningful evaluation of safety margins or species differences in pharmacological and toxicological effects than a simple dose comparison
Calculation of Safety Factors

\[ SF = \frac{\text{non toxic plasma concentration in animals (cmax or AUC)}}{\text{max. therapeutic plasma concentration in man (cmax or AUC)}} \]
Which safety factors are needed?

- There are no fixed rules. The safety factors depend on:
  - Severity of findings (transient, progressive, irreversible)
  - Possibilities to monitor the toxicities in humans by non-invasive methods (easy-to-monitor vs. difficult-to-monitor vs. impossible to monitor)
  - Slope of the dose-response curve
  - Intended indication (risk vs. benefit)
The take-home message is

- Kinetic and toxicokinetic information is important for the extrapolation of animal data to humans.

- Safety factors should be calculated on the basis of plasma concentrations (exposure).

- There are no fixed rules which safety factors are needed (depends on the nature of the toxicological findings).
Why do we investigate kinetics of Drugs?

• Drug effects (wanted + unwanted) depend on the concentration at the site of action

• The variability of drug concentrations in the body has an impact on efficacy and on safety

• Pharmacokinetic drug interactions (e.g. inhibition of metabolizing enzymes) can be clinically relevant