An Introduction to Pharmacokinetics

Pete Webborn

1. Part 1 - Basics
2. Part 2 – A more detailed look at key parameters
3. Part 3 – Prediction of human PK

Glossary

https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1365-2885.2001.00340.x

An Introduction to Pharmacokinetics

Pete Webborn

1. Part 1 - Basics
    1. Drug exposure - The key to understanding efficacy and safety
    2. Measuring exposure in safety and efficacy studies
    3. Pharmacodynamics and PKPD – basics. Safety margins
    4. The key physicochemical properties of drugs
    5. Key parameters – Clearance and volume of distribution
    6. Drug Absorption
    7. Plasma protein binding

If a successful drug is all about its Efficacy and Safety, How does DMPK contribute?

"Poison is in everything and nothing is without poison. The dosage makes it a poison or a remedy" (Paracelsus – 16th century)

20th century update - It is the exposure, not the dose that matters!

Exposure to What? Where? How much is needed? how much is too much? In Whom? With what co-meds? What dose will give the right exposure?

(***21st century update*** - It's an individual's genetics that can be defining)

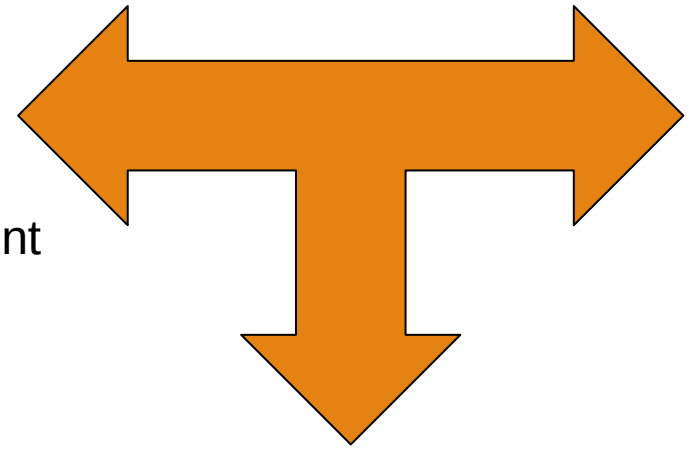
These are the questions that DMPK seeks to:

1. Answer (in Development)
2. Help design against (in Discovery) "Good DMPK properties"

The role of PK in drug discovery

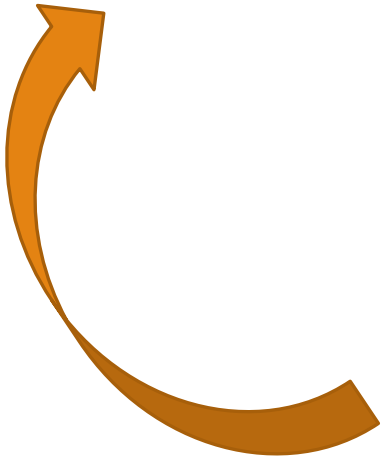
PKPD / TKTD
- Exposure-response
- Target validation
- Biomarker development
- PKPD Model development

Translation to man

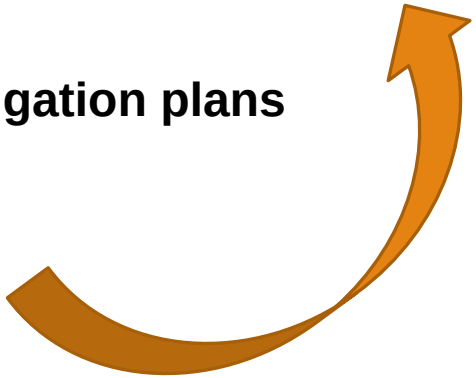


Compound Design and Selection
- Understand disposition
- QSAR /in silico tools
- Improve properties
- Human PK prediction

Translation to man



Opportunity/ Risk assessment / mitigation plans
- Predictions of human PK and PKPD
- Estimation of likely safety margins
- Project investment decisions
- Design of clinical plan

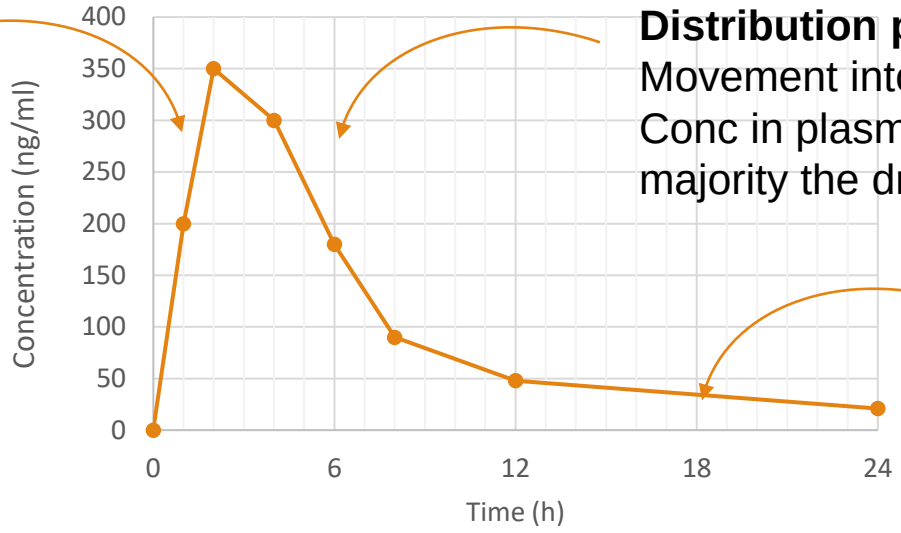


What happens after a oral dose is taken?

Plasma analysis

“Absorption phase”
Dissolution
Permeation of gut wall

Fast absorption = high and early Cmax



Distribution phase
Movement into tissues
Conc in plasma may decline, but majority the drug is still in the body

Elimination Phase
Drug removed from body by metabolism, renal excretion, biliary excretion

Refers to the “predominant” fate of molecules at that time

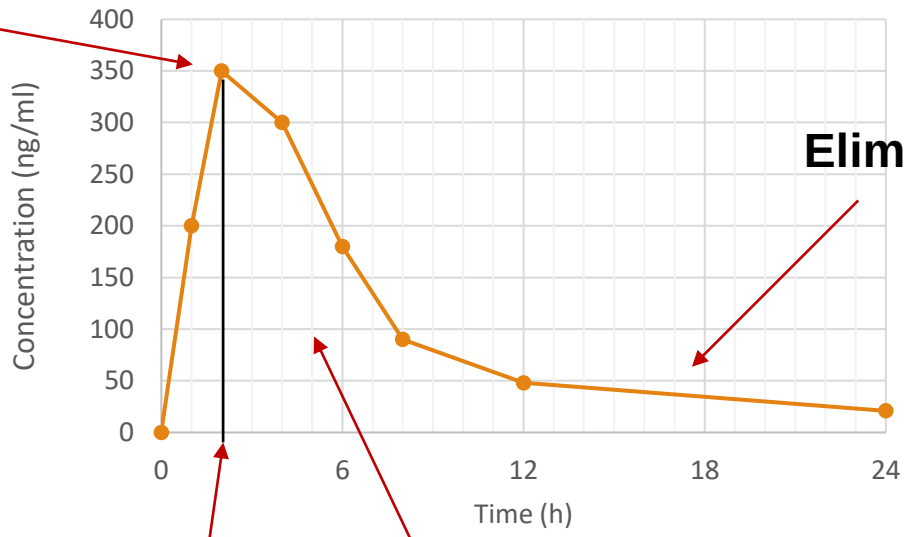
Curves are best described by the parameters that we use to characterise them

Aim of these talks - Understand the Parameters, what is a high and a low number, and how the physicochemical properties of a molecule govern its PK

What happens after a oral dose is given?

What can be usefully be measured?

Maximum concentration (Cmax)



Elimination half-life

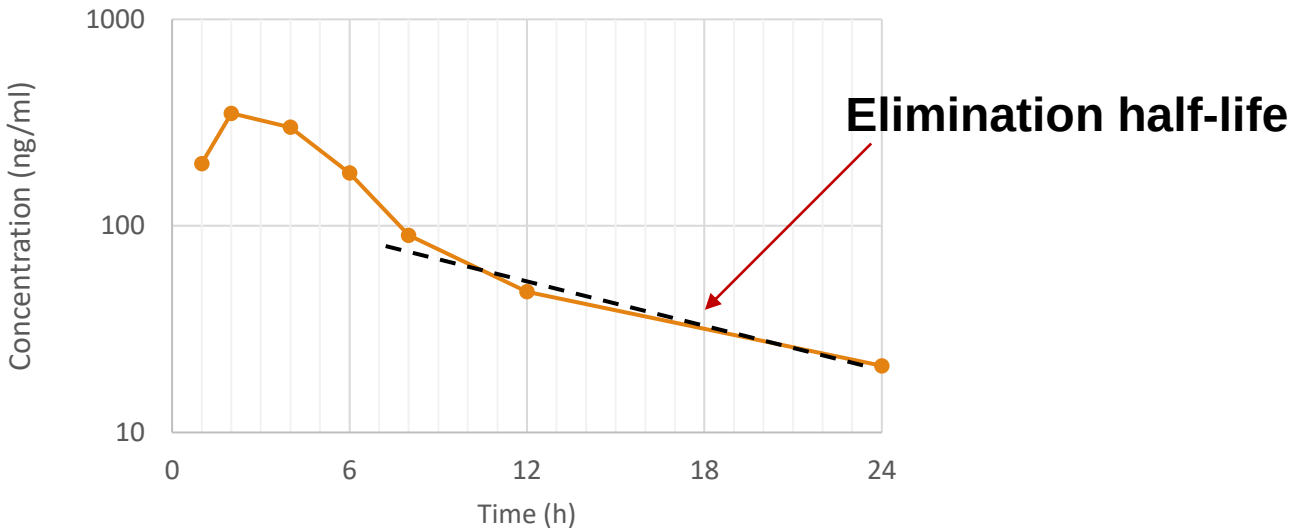
Time of maximum Concentration (Tmax)

Area under the curve (AUC)
Mean residence time (MRT)

"Exposure" is usually characterised by the plasma AUC (or Cmax)

What happens after a oral dose is given?

Semi-logarithmic graphs



- **Useful for selecting points to use to estimate the half-life**
- **Useful for comparing PK profiles at different dose levels**
  - **NOT AUCs**

Two kinds of Pharmacokinetic Parameters

Parameters we measure – tell us about exposure

"Area under the curve" - AUC
Maximum concentration - Cmax
Time of ,maximum concentration - Tmax
Half-life
Bioavailability

Parammeters we calculate that have physiological relevance - they allow us to *link a compound's structure to its biological interactions*

Clearance /Clearance mechanisms
Volume of distribution
Absorption

A really important distinction

Pharmacokinetics

Can be complex - But only three main processes!

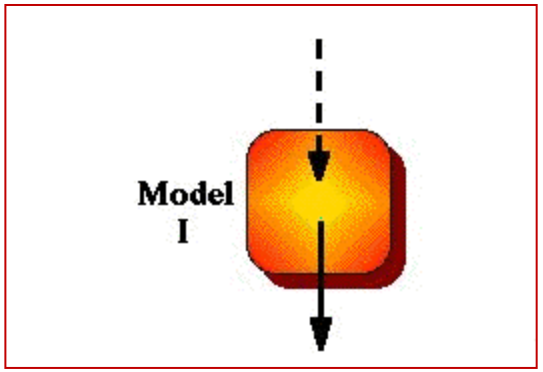
Absorption

Kinetics of crossing barriers
All about permeability and solubility*

The key question – Why is absorption incomplete?
A lot can be understood from Physicochemical properties
LogP/LogD, pKa, polar surface area, rotatable bonds

Elimination (renal, metabolic etc)

Reaction Kinetics
(Pseudo) first order processes
Rate of elimination proportional to concentration
Most usefully described as "clearance" –more later!



Distribution

Mainly about the relative affinity of a drug for tissue v plasma

(barriers are a specific case – rare / predictable)

Bioanalysis - Toxicokinetics

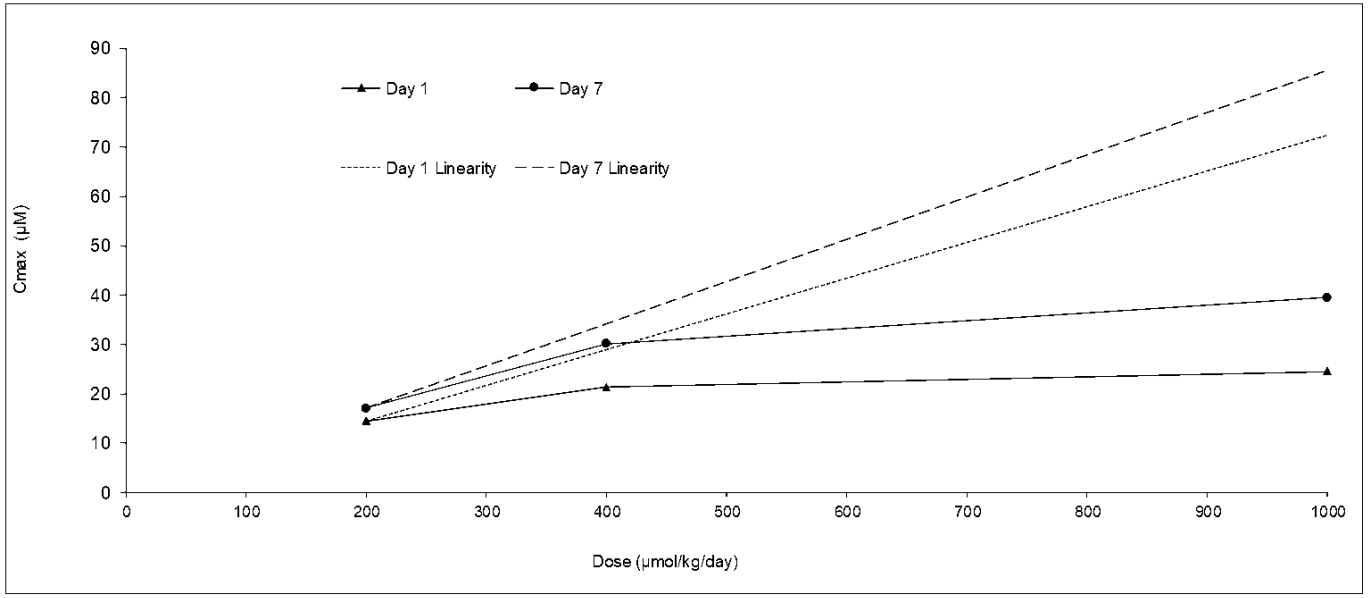
Analysis batch data

Sample Name	Filetype	Vial Position	Calc.Conc.	Accuracy
Blank	Blank	1	N/A	N/A
Blank	Blank	2	N/A	N/A
Double blank	Blank	3	N/A	N/A
STD.1	Standard	4	0.201	101
STD.3	Standard	5	0.978	97.8
STD.5	Standard	6	5.02	100
STD.7	Standard	7	20.9	104
STD.9	Standard	8	99	99
STD.10	Standard	9	201	101
Blank	Blank	10	N/A	N/A
Blank	Blank	11	N/A	N/A
LoQC	Quality Control	12	0.787	131
MidQC	Quality Control	13	10.5	105
HiQC	Quality Control	14	164	102
Blank	Blank	15	N/A	N/A
Blank	Blank	16	N/A	N/A
Group2_Dose100_Day1_04_1h	Unknown	17	51.4	N/A
Group2_Dose100_Day1_04_6h	Unknown	18	70.5	N/A
Group2_Dose100_Day1_04_24h	Unknown	19	3.11	N/A
Group2_Dose100_Day1_07_1h	Unknown	20	88.9	N/A
Group2_Dose100_Day1_07_6h	Unknown	21	74.8	N/A
Group2_Dose100_Day1_07_24h	Unknown	22	2.99	N/A
Group4_Dose100_Day1_11_1h	Unknown	23	101	N/A
Group4_Dose100_Day1_11_6h	Unknown	24	82.5	N/A
Group4_Dose100_Day1_11_24h	Unknown	25	41.4	N/A
Group4_Dose100_Day1_12_1h	Unknown	26	99.1	N/A
Group4_Dose100_Day1_12_6h	Unknown	27	51.7	N/A
Group4_Dose100_Day1_12_24h	Unknown	28	8.26	N/A
Group4_Dose100_Day1_13_1h	Unknown	29	55.4	N/A
Group4_Dose100_Day1_13_6h	Unknown	30	42.9	N/A
Group4_Dose100_Day1_13_24h	Unknown	31	5.59	N/A
Blank	Blank	48	N/A	N/A
Blank	Blank	49	N/A	N/A
LoQC	Quality Control	50	0.675	112
MidQC	Quality Control	51	10.6	106
HiQC	Quality Control	52	153	95.9
Blank	Blank	53	N/A	N/A
Blank	Blank	54	N/A	N/A
STD.1	Standard	55	0.199	99.3
STD.2	Standard	56	0.505	101
STD.4	Standard	57	2.01	101
STD.6	Standard	58	10.1	101
STD.8	Standard	59	49.4	98.8
STD.10	Standard	60	192	96
Blank	Blank	61	N/A	N/A
Blank	Blank	62	N/A	N/A

Key judgement - Is the data likely to be describing the true profile?

Results – What are we looking for?

What is the relationship between dose and exposure?



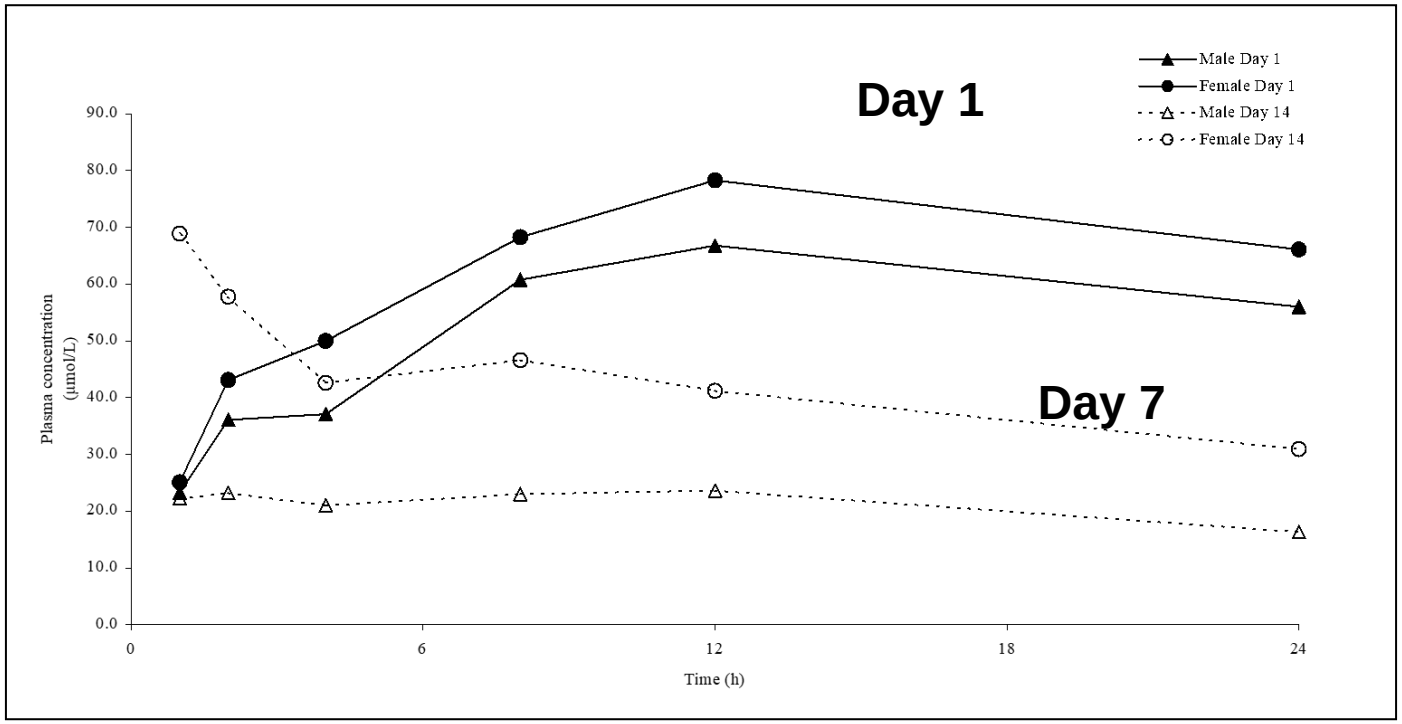
Conclusion

An increase in exposure with dose was observed between 200 and 400 mg/kg, however no increase in exposure was observed following dosing at 1000 mg/kg

All increases were less than dose-proportional

Results – What are we looking for?

Is there a difference between Day 1 and Day 7?



Following multiple dosing exposure as assessed by C_{max} and $AUC_{(0\text{-}24)}$ was decreased following multiple dosing when compared with a single dose.

Pharmacodynamics

The branch of pharmacology concerned with
the action of drugs on the physiology or pathology of the body

Deals with the time course of effects, and leads to mechanistic understanding that influences how doses / dose regimens are planned

Dose response

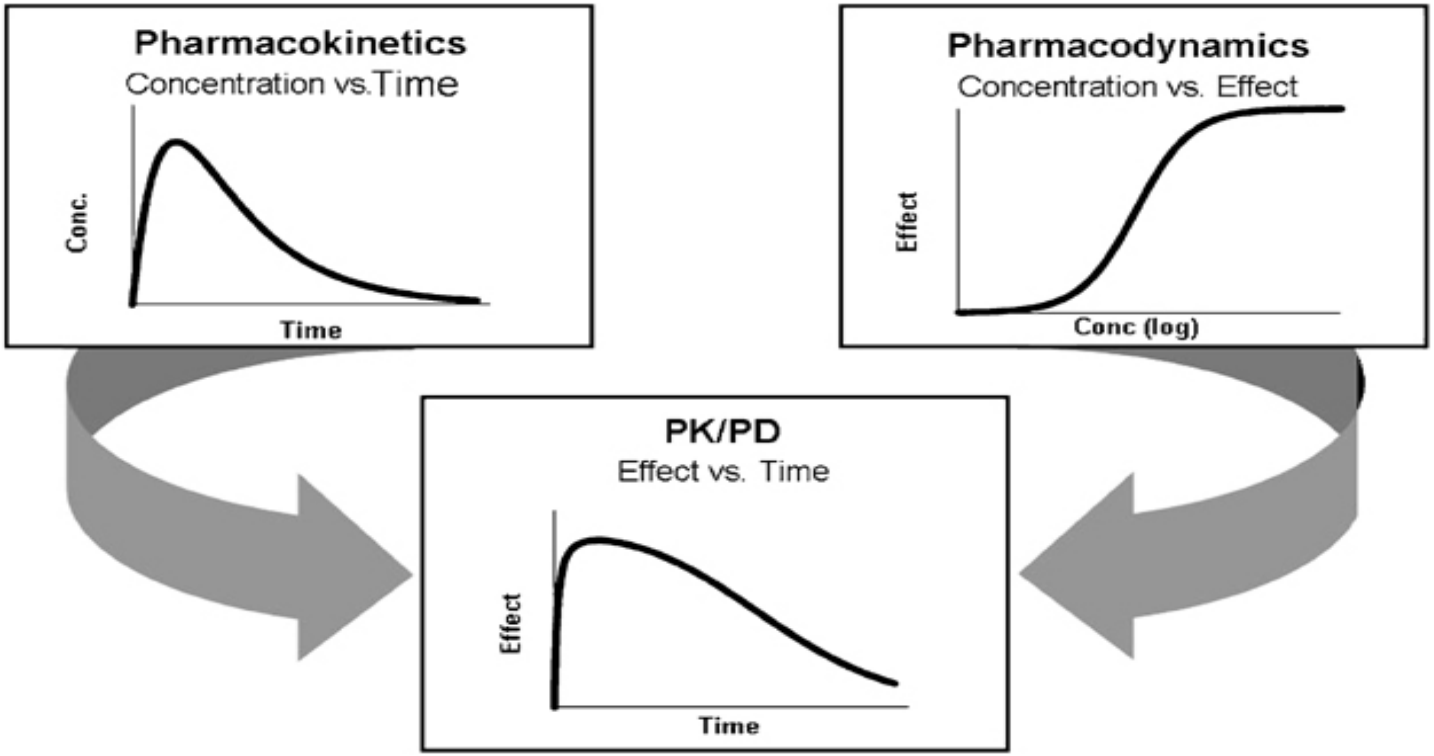
Concentration response

Concentration – time – response (mathematical models)

PK-PD models

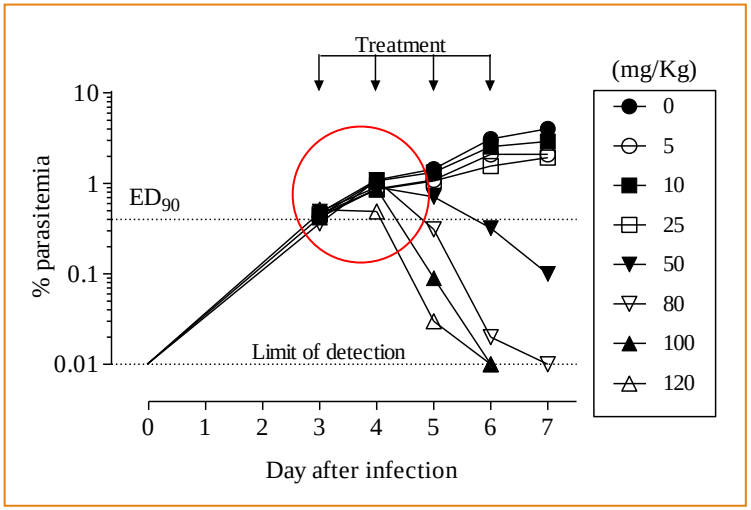
A Basic overview

A Pharmacokinetic model that describes exposure is linked to a Pharmacodynamic model => effect over time

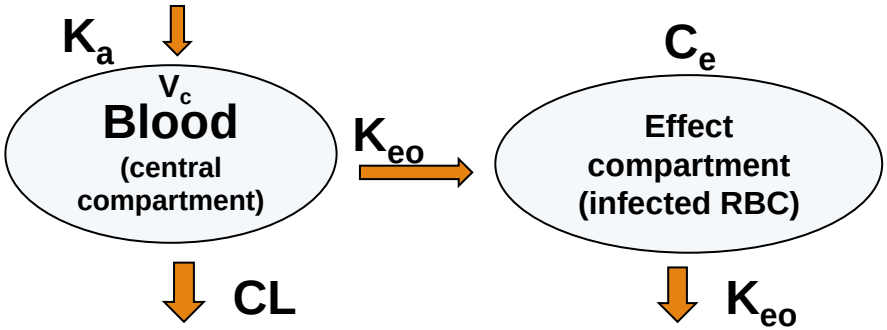


e.g. Emax model

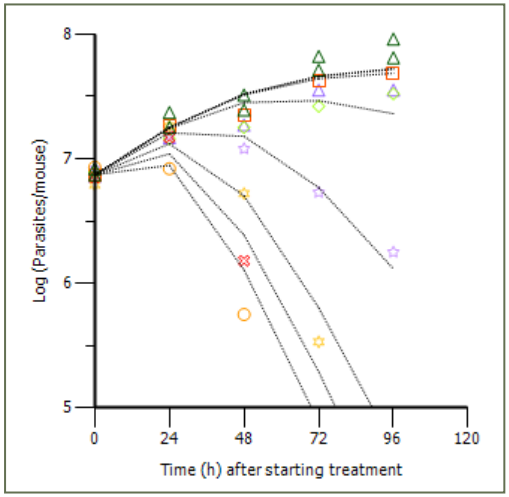
PK-PD modelling: Fit to dose response in the *P.falciparum* SCID mouse model



Effect compartment was introduced to model the delay observed in efficacy



Parameter	Unit	Estimate	%CV
K_{gro}	1/h	0.046	-
P_{max}		7.756	9
K_{kill}	1/h	0.191	159
CL / F	mL/min/kg	94	25
Vc / F	L/kg	269	5
K_{e0}	1/h	0.032	240
EC_{50}	µM	0.644	148



MMVSola

Predicting doses from PK and *in vitro/in vivo* PD

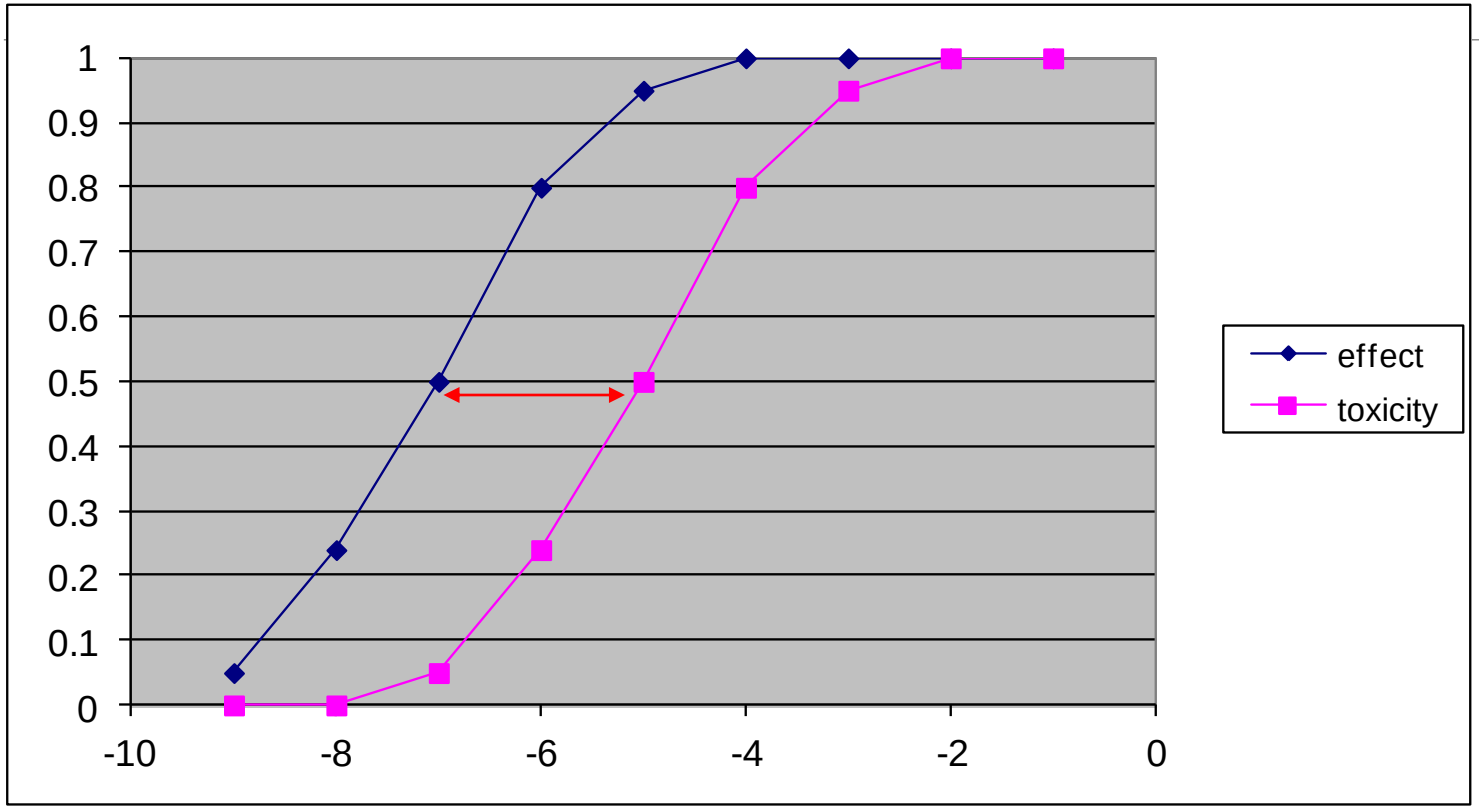
Safety Margins

$$\text{Margin} = \frac{\text{Animal Exposure* at No adverse effect dose}}{\text{Human Exposure* at a given dose}}$$

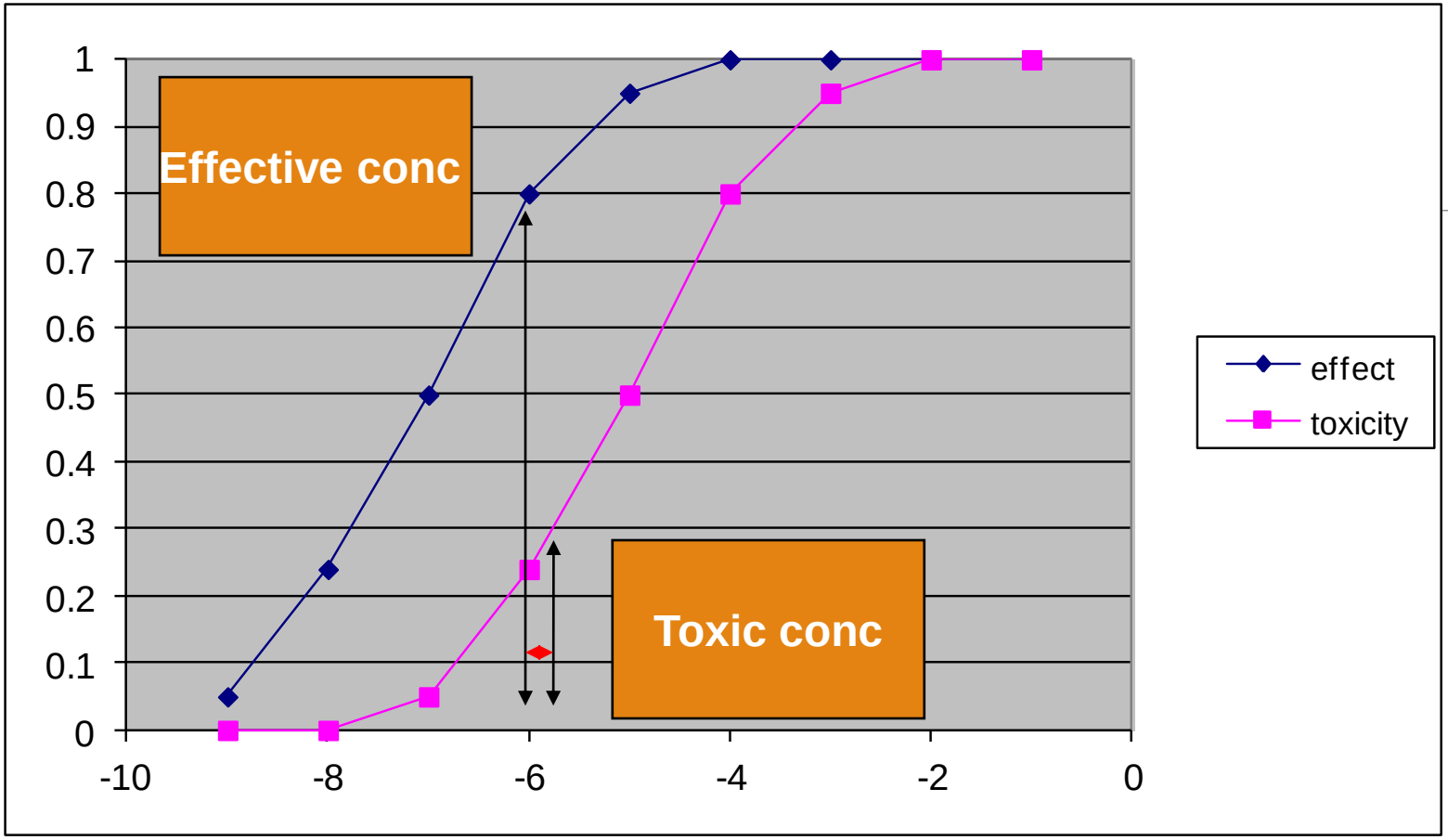
* AUC or Cmax, free or total (regulators tends to consider “worst case”)

...But not all margins are the same (and what might seem large can reduce rapidly upon further consideration)

Pharmacological Safety Margins – Theory
In vitro data (underpins early decision making)

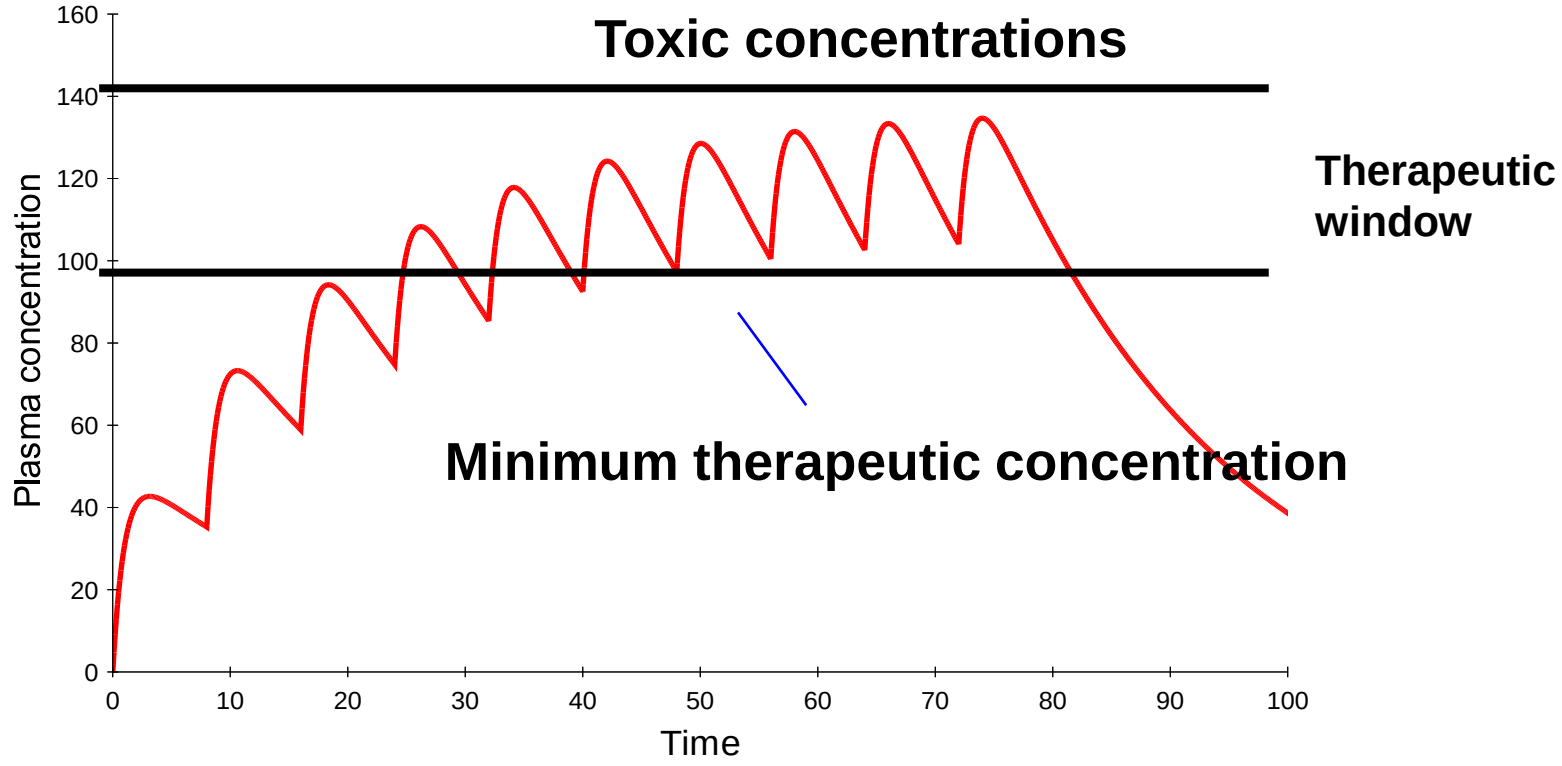


Target **pIC_{50} = 7, 0.1μM**
hERG **pIC_{50} = 5, 10μM**
Implies a 100 X margin



1. **Different levels of occupancy required for efficacy and toxicity**
2. **E.g. Efficacy related to trough Conc at 24 h, Safety is Cmax dependent**
3. **Then consider Inter-subject variability (PK and PD)**

Pharmacokinetic optimisation
Efficacy at acceptable doses/dose intervals without side effects

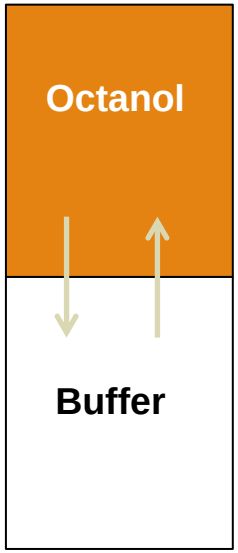


Low Dose <100 mg - Potent + cleared slowly + bioavailable
Long dose interval - Long half-life*
Low peak to trough ratio - Long half-life

Physico-chemical properties and PK

LogP and LogD

- LogP is a measure of lipophilicity
- Log P is the octanol-water partition coefficient of the unionised drug



- A ratio of 100:1 = a LogP of 2
- A ratio of 1:1 = a LogP of 0

- $LogD_{7.4}$ is the octanol-water partition coefficient at pH 7.4

Because it accounts for ionisation (and is close to physiological pH) LogD tend to be more useful in biological systems

- *"Lipophilic" in the drug world - LogD >2*
- *"Polar" in the drug world - LogD <1*

Physico-chemical properties and PK

pK_a - Ionisation constant

Acids dissociate by releasing a proton

$$HA \rightleftharpoons H^+ + A^-$$

$$K_a = \frac{[A^-][H^+]}{[HA]}$$

Useful:
Carboxylic acid pK_a ~4.5
simple amine pK_a ~9

pK_a is the negative log of K_a

The bit to remember....The pK_a is the pH at which the compound is 50% ionised

This is the same for bases $B + H_20 \rightleftharpoons HB^+ + OH^-$

The other bit to remember.....For every log unit the pK_a is away from physiological pH, the ratio of ionised to unionised changes 10 fold

At pH 7.4

a base of pK_a 7.4 is 50% charged
a base of pK_a 8.4 is 90% charged
a base of pK_a 9.4 is 99% charged
a base of pK_a 6.4 is 10% charged

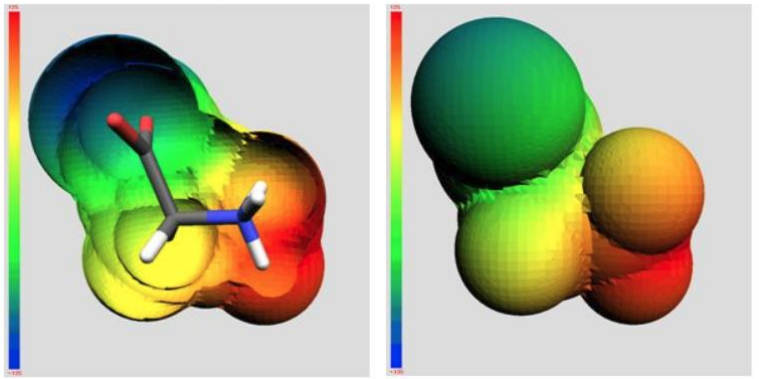
an acid of pK_a 7.4 is 50% charged
an acid of pK_a 6.4 is 90% charged
an acid of pK_a 5.4 is 99% charged

Physico-chemical properties and PK

Polar Surface area (PSA)

The PSA is defined as the molecular surface that stems from the polar atoms (usually nitrogen and oxygen atoms and their polar hydrogen atoms)

- A measure of ability to form hydrogen bonds.
- Linked to ability to pass through membranes
- PSA > 80-100Å^2 an inflection point
- Can be important in drug:
  - Intestinal absorption
  - Brain penetration
  - Cell penetration
- Too high a PSA = poor membrane permeability
- Likely to correlate with lipophilicity



Solubility
…with permeability –the key to good absorption

- Kinetic solubility – Screening assay (From a DMSO stock sometimes)
  - Typically - 0.1M phosphate buffer pH7.4
  - Solubility of the fastest dissolving/precipitating species
  - Rough Guide: >100ug/mL Probably ok, < 10 ug/mL Probably a problem
- Thermodynamic solubility – Equilibrium solubility of all species
  - Not that common/useful in discovery?
- More useful: Simulated biological fluids
  - Fassif / Fessif Simulated fasted or Fed intestinal fluids
  - Almost always higher that screening solubility
- Note:
  - Anti-malarials tend to have large doses so solubility is important
  - Final salt/polymorph may be different to discovery batches
  - Later/more crystalline materials may have worse solubility
  - Expensive formulation options less available to anti-malarials?
- **Ignore solubility issues at your peril!**

Pharmacokinetics

Oral data gives little information about the properties of a compound...

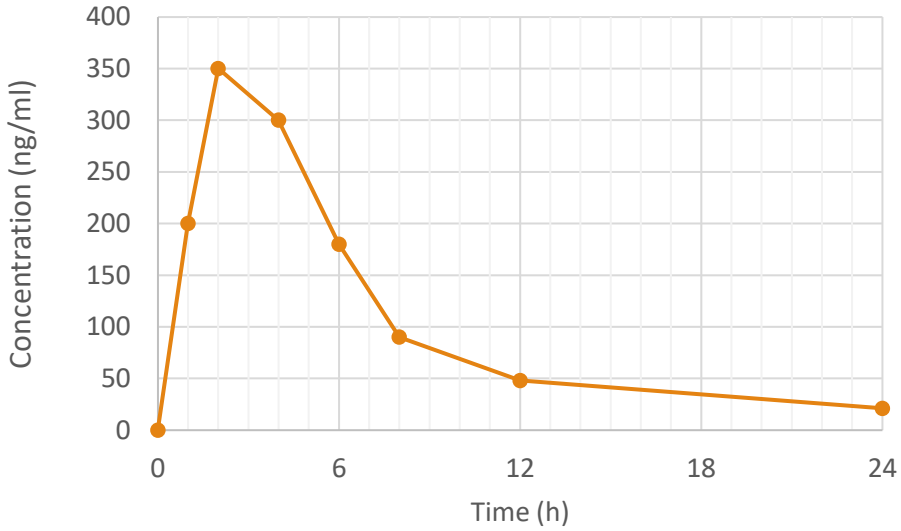
Is the compound well absorbed ?

Is it highly bioavailable ?

How well does it distribute in the body?

Is this a high hepatic extraction compound?

Is this what would have been predicted from in vitro/ in silico data?



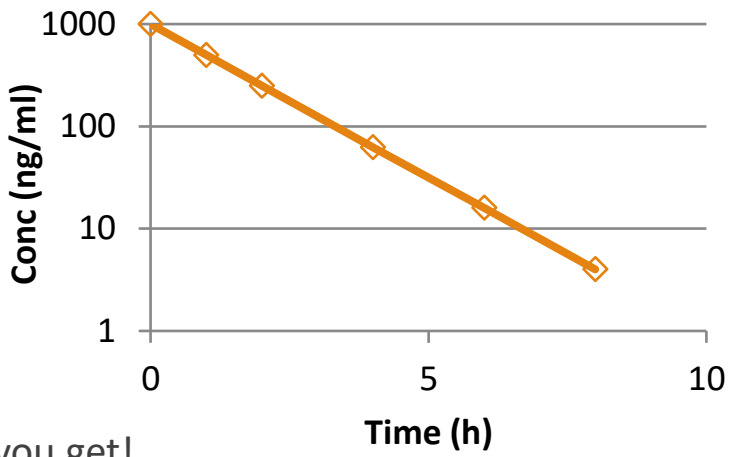
- **Oral dosing is the key to real understanding**
- **Two key parameters are obtained after an iv dose "Clearance" and "Volume of distribution"**

The Magic of AUC!

Double the dose -> double the AUC

This means ---->

Dose (ng) / AUC (ng min /ml) = a constant



Units of the constant? ml/min ! Flow!

Definition of the constant – "Clearance"

"A scaling factor between the dose you give and the AUC you get!

"Clearance" = Dose /AUC

Also

"Clearance" (mL/min) x plasma concentration (ng/mL) = rate of elimination (ng/min)

Importance of units in PK!

Low clearance compounds have a higher AUC at a given dose

Divided into "metabolic clearance", "renal clearance" etc

More in Next lecture

Volume of Distribution

"The Volume in which the drug appears to be dissolved!"

Compound A Dose 1 mg/kg Co = 1000 ng/mL

To estimate V...

Give a set dose, estimate Co in plasma.....
……..calculate V (L/Kg) from

(Conc = Mass/Volume)+

Or

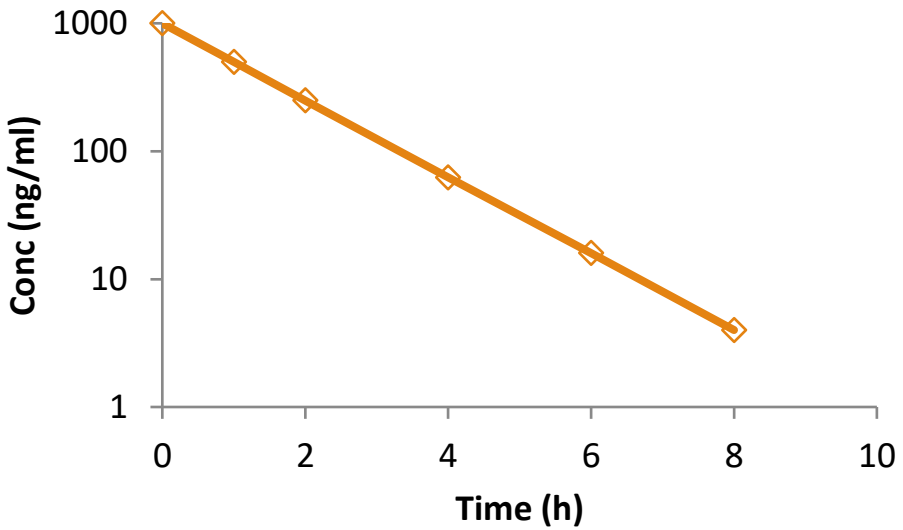
(Volume = Mass/Conc

Co = 1000 ng/ml.....V= 1.0 L/Kg

Co = 10,000 ng/ml.....V= 0.1 L/Kg

Co = 100 ng/ml.....V= 10 L/Kg

How can a drug distribute into 10L/Kg!!!!!



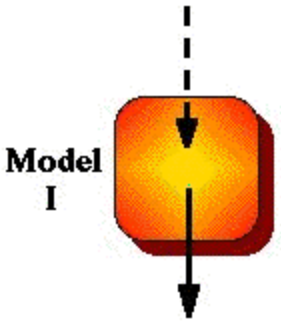
Volume of distribution is a measure of the relative affinity for plasma and tissues

Half-life

Pharmacokinetics are governed by input and output rates and volumes of distribution

Half-life is dependent upon 2 parameters

- **Efficiency of elimination processes (Clearance mL/min/kg)**
- **Volume of distribution of drug (L/kg)**



$$T\frac{1}{2} = \frac{0.7\ V}{CL}$$

Drugs with higher volumes of distribution and lower clearances rates have longer half-lives

What is “Oral Bioavailability” ?

The fraction of an oral dose reaching the systemic circulation as intact drug

(nothing to do with the target tissue)

Abbreviated to F or F%

This can also be expressed

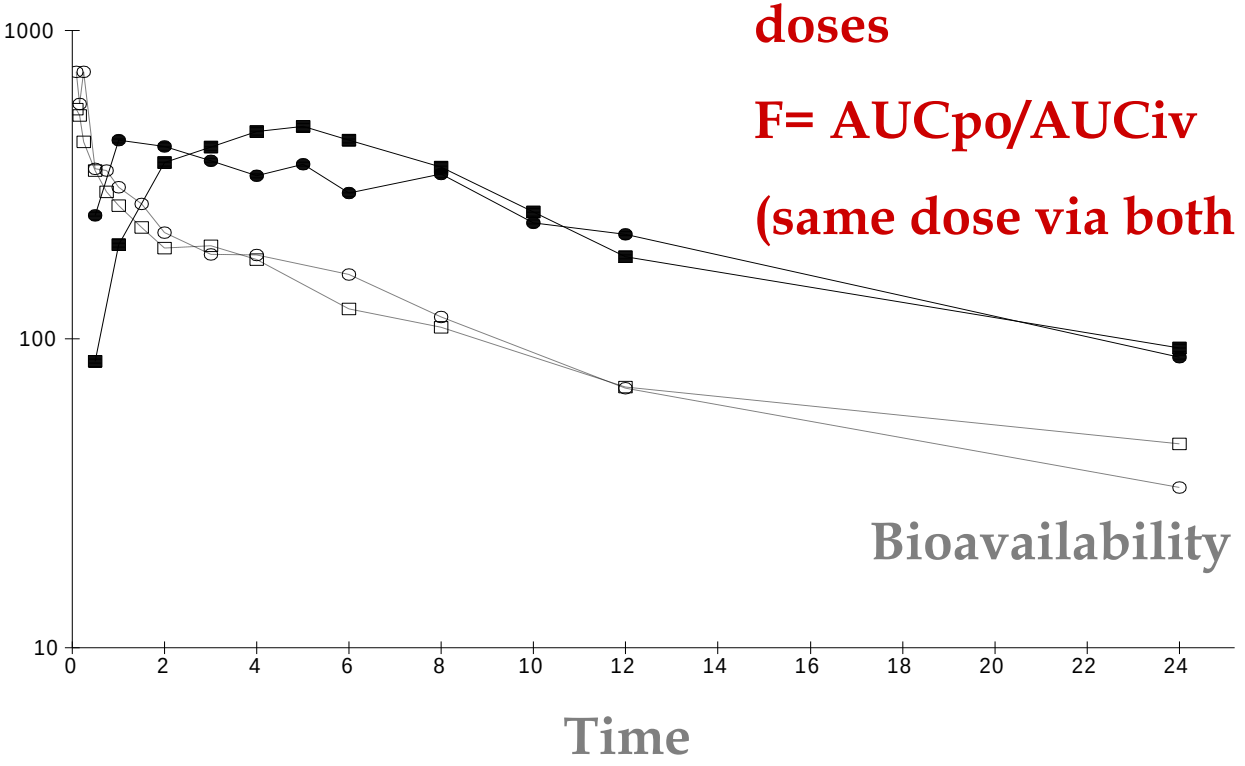
$F = AUC_{PO}/AUC_{IV}$ if iv and po doses are the same

How is Bioavailability estimated ?

Measure AUC after iv and po doses

F= AUCpo/AUCiv

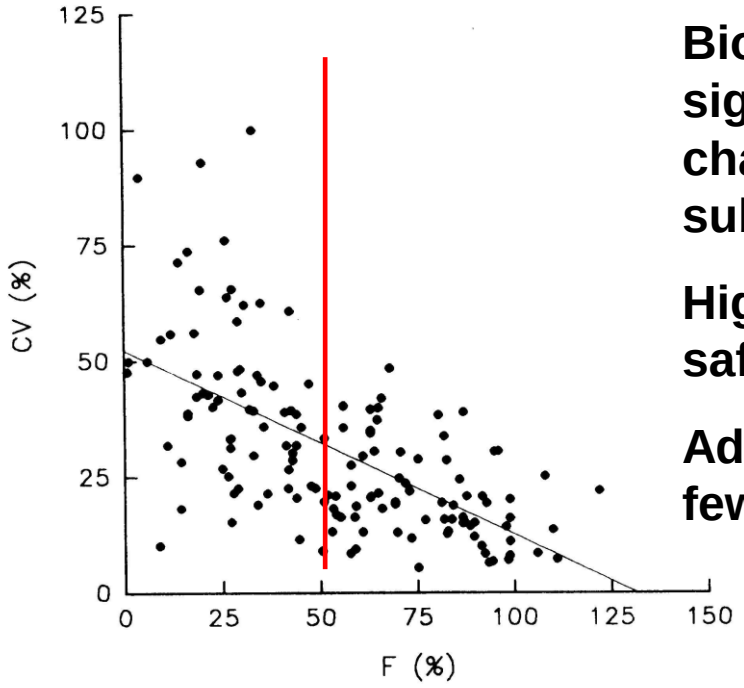
(same dose via both routes)



Bioavailability = 40 %

Why is Bioavailability a key parameter?

- **Low bioavailability increases dose size**
- **Low bioavailability increases intersubject variability**



Bioavailability of <50% significantly increases chances of high inter-subject variability

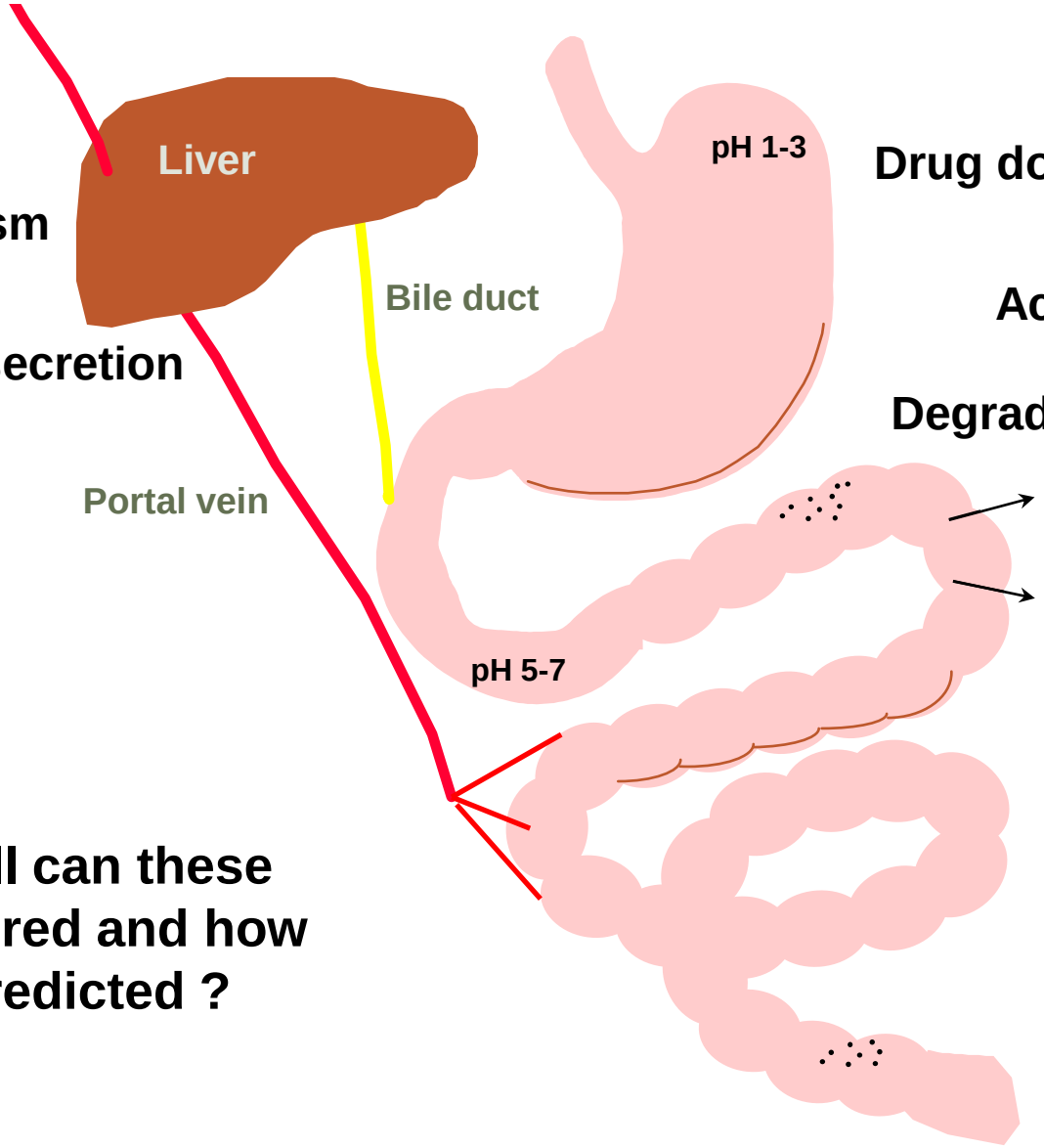
High variability impacts safety margins.

Adverse events in just a few patients kill drugs

Hellriegel et al (1996)

Clin Pharm Ther 60(6) 601-606

What factors can limit bioavailability ?



Hepatic metabolism

Biliary secretion

Drug does not dissolve

Acid unstable

Degraded by gut enzymes

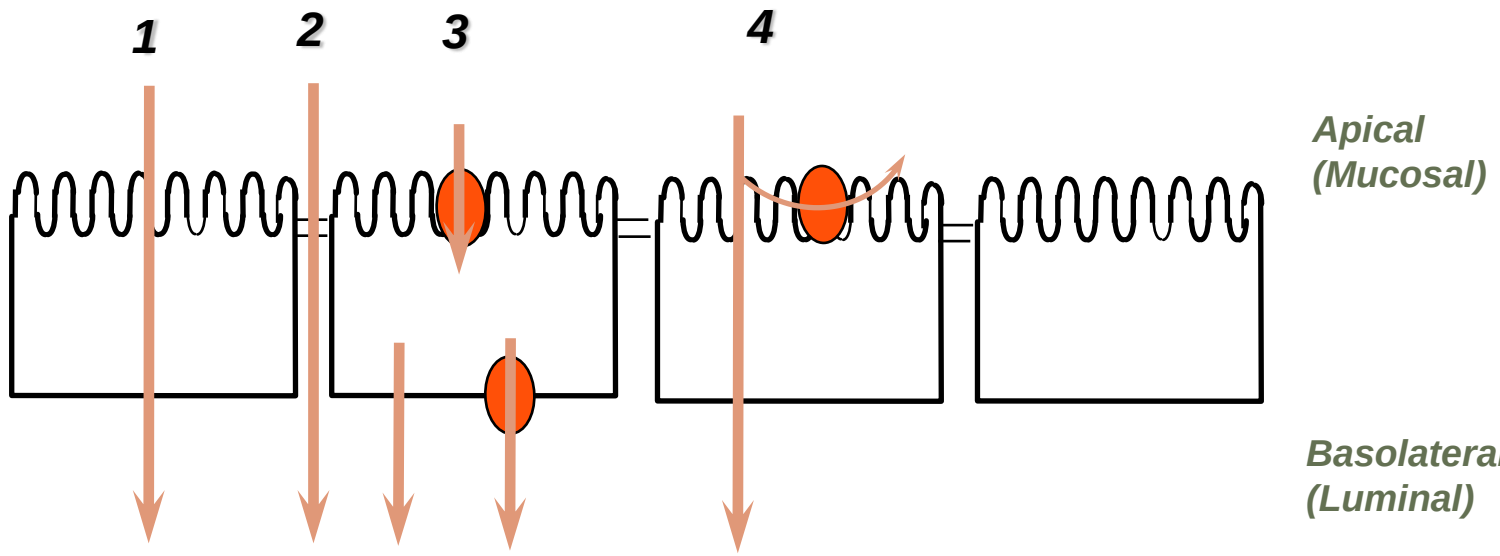
Efflux substrate

Gut Wall metabolism

Does not permeate gut wall

How well can these be measured and how well predicted ?

Mechanisms of Permeability

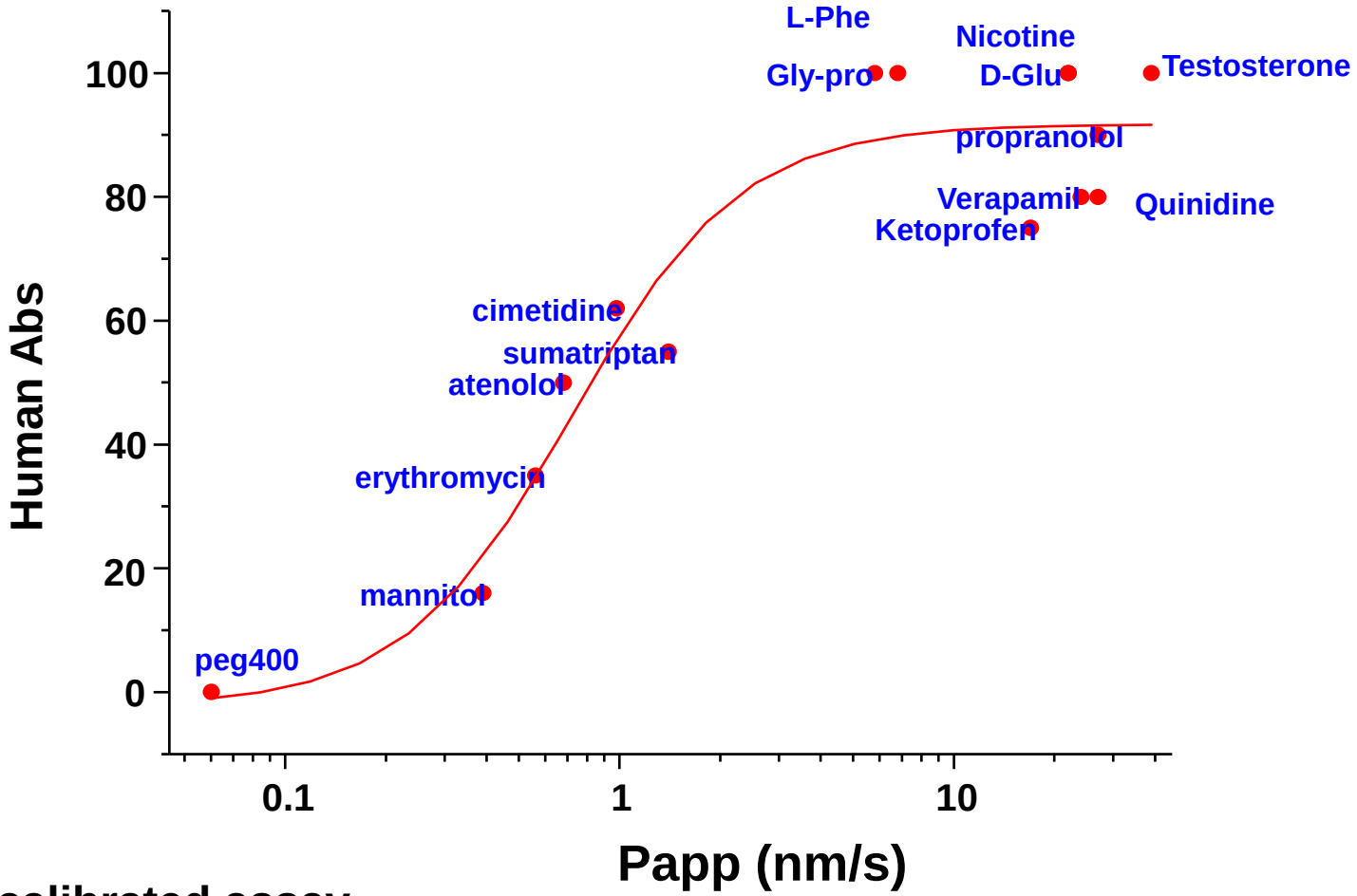


1- Transcellular
2 - Paracellular
3 - Carrier Mediated
4 - Efflux

Transcellular absorption by far the most important mechanism for anti-malarials (lipophilicity)

Efflux usually saturated as doses >200mg……

Calibration of Caco-2 assays

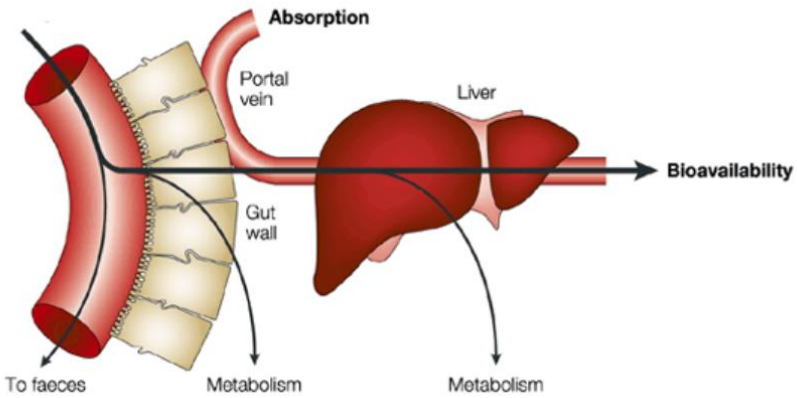


- Use a calibrated assay
- Establish whether low permeability is due to poor “intrinsic” or “lipoidal” permeability or to high efflux

How to estimate the Fraction absorbed

The fraction absorbed is the bioavailable fracture + the fraction eliminated in the liver and gut between entering the gut wall and reaching the systemic circulation

If Bioavailability is 60% and 20% is metabolised in liver “First-pass” then absorption must be 80%



“Hepatic extraction”

$$Hepatic\ extraction \quad E_H = \frac{CL_H}{Q_H}$$

$$Hepatic\ availability \quad F_H = 1 - \frac{CL_H}{Q_H}$$

How to estimate the Fraction absorbed

Bioavailable fraction = fraction absorbed x fraction not metabolised in the liver or gut:

$F = F_a \times F_H \times F_g$

However, for low clearance compounds the fraction metabolised in the gut wall is small

- Gut wall metabolism only a factor when hepatic metabolism is high (CYP3A4/5 and UGTs) – Antimalarial candidates should not have high clearances!

As, $F = Fa \times F_H$ or $Fa = F/F_H$ and as $F_H = 1 - E_H$

$$Fraction\ absorbed = \frac{F}{1 - \frac{CL_{H,Blood}}{Liver\ blood\ flow}}$$

NOTE: Blood clearance not plasma clearance. Especially important for anti-malarials!

NOTE: Hepatic clearance = Total clearance - non-hepatic clearance

Example: Rat Fraction absorbed

	Compound A
Plasma Clearance (mL/min/kg)	98
Vss (L/kg)	83
Half-life (h)	12
Bioavailability (%)	89
B:P	9
Blood Clearance (mL/min/kg)	11
Fraction Absorbed (%)	103

Species	Q_h mL/min/kg
Mouse	126
Rat	77
Dog	56
Human	20.7

$$F_{abs} = \frac{F}{1 - \frac{CL_{Blood}}{Liver\ blood\ flow}}$$

$$103\% = \frac{89\%}{1 - \frac{11}{77}}$$

How to predict the fraction absorbed in humans?

Use animal data

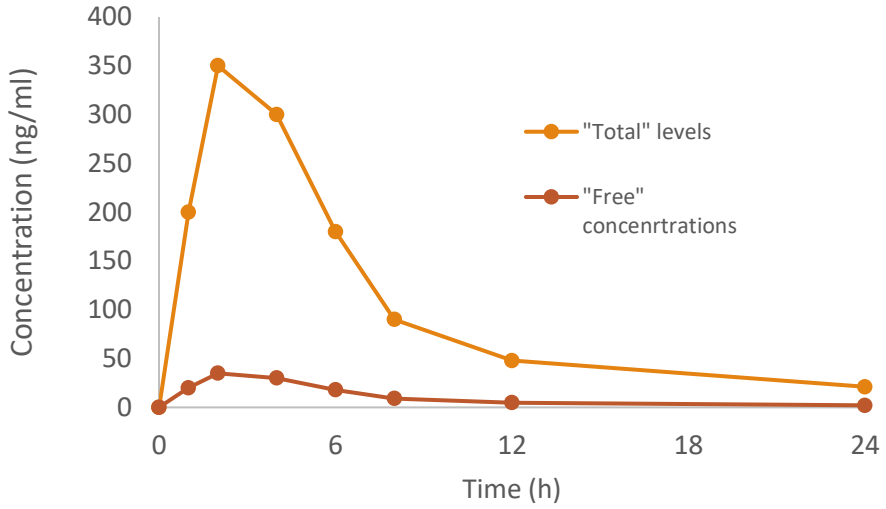
- Formulations and doses must be relevant for human

Use in vitro methods

- Permeability and solubility
- Tools Like Gastroplus will do this
- Good for identifying risks for good absorption rather than quantifying?

Important – In diseases like malaria where doses are large, projects should not be bringing forward compounds with poor absorption (<90%)

Plasma protein binding



Simplistically - if a compound is 90% bound, free levels are 10% of what you measure in vivo (Total levels)

Measured by equilibrium dialysis in vitro

High binding (>99.5%) can be a challenge to measure accurately

Protein binding does not need to be optimised, high or low binding just different PK properties

Plasma protein binding – Key points

Drugs can be 99.99% bound to plasma proteins, but it is a rapid equilibrium

Cells, enzymes and drug targets are in equilibrium with the free drug

[Intracellular free] assumed to be same as [plasma free] at equilibrium (unless transporters involved, pH effects)

Unbound fraction is the relevant parameter:

- A compound that is 95% bound, has twice the proportion free as one that is 90% bound

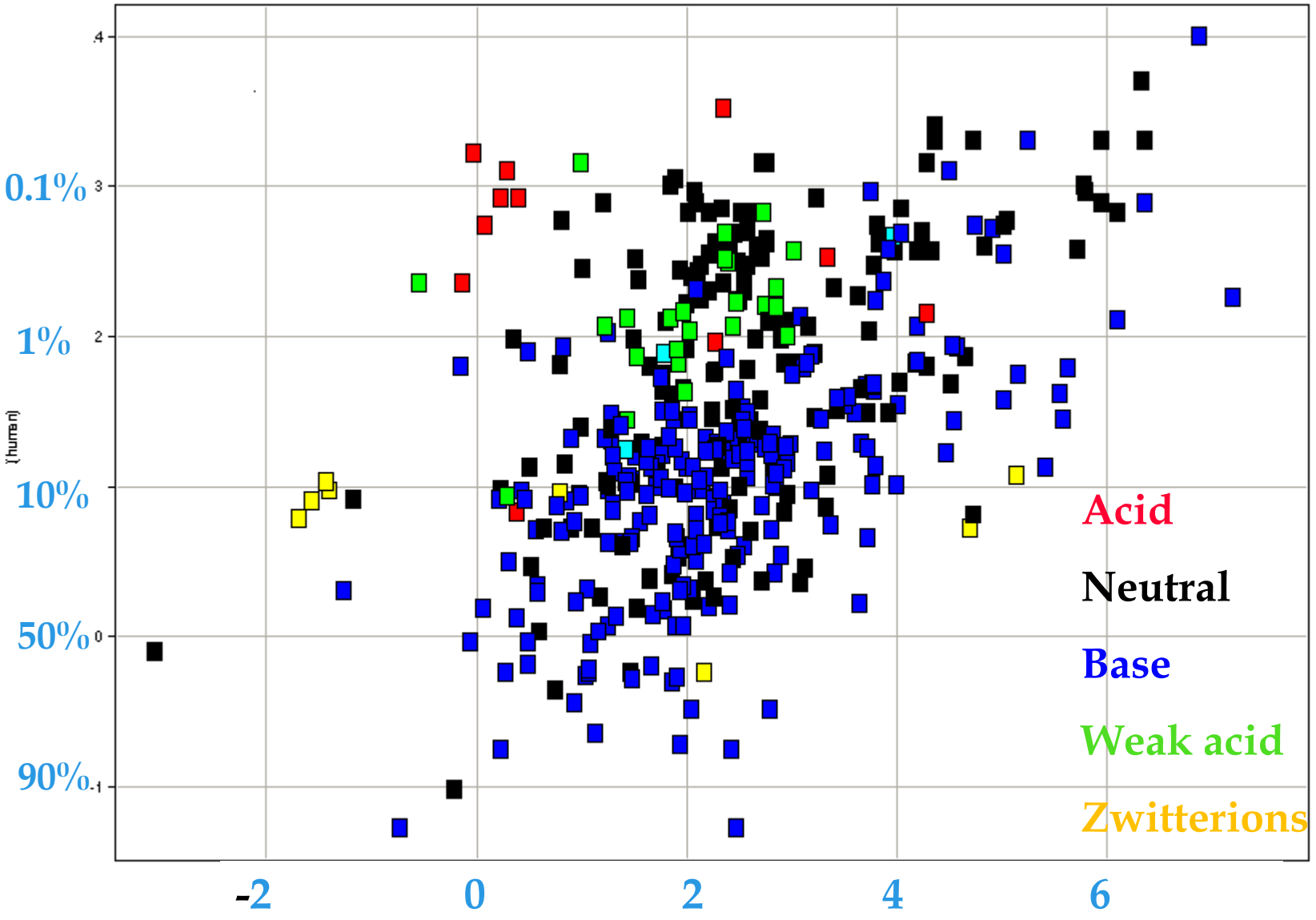
Albumin is the major source of binding of acidic and neutral compounds – A very high capacity system (Albumin is present in plasma at 600uM)

Bases bind to α1-acid glycoprotein (~15uM, but can vary in some disease states)

Lipophilicity and charge are the key drivers

Note: *high ppb makes total plasma levels high, not free levels low!!?!!* ***More later!***

Plasma Protein Binding and lipophilicity



Summary

1. PK to describe exposure
2. PK to link to physiology and optimisable properties
3. PK is driven by a molecule's physicochemical properties
4. PK to understand efficacy / to build PKPD models
5. Half-life - a function of the volume of distribution and the clearance

   $$T1/2 = 0.693\ V / CL$$

6. Bioavailability is limited by incomplete absorption and "first-pass" metabolism
7. Drug effects are driven by "free concentrations"