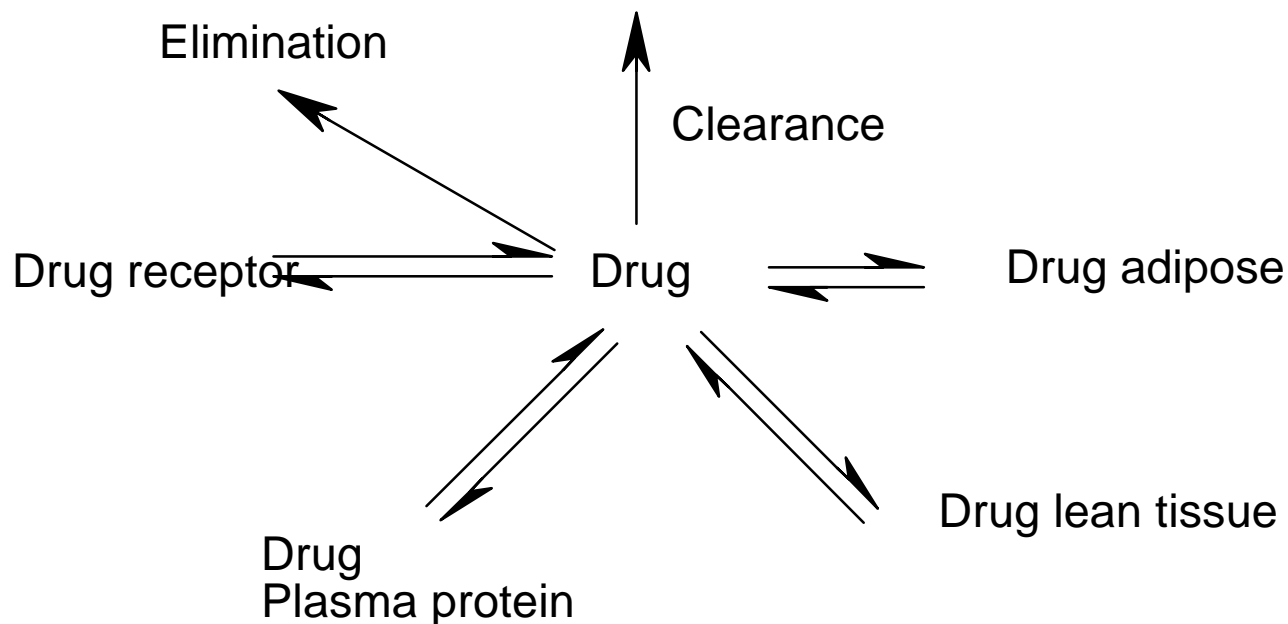


Statistical Ambiguities in the use of Unbound Pharmacokinetic parameters in QSAR's

Andy Davis, David Salt, and Peter Webborn

Coupled equilibria



- Equilibria and irreversible steps are coupled
- in-vivo Cl and Vss are
 - “compound” properties $Cl = Cl_r + Cl_h + Cl_{\text{other}}$
 - secondary parameters - modulated by protein binding

What chance is there of extracting meaningful QSARs ?

Approaches to PK-QSAR

- From in-vivo data - correct for protein binding
 - calculate unbound Cl/ unbound Vss
 - primary variables ideal for qsar

Well stirred model

$$V_{uss} = \frac{V_{ss}}{f_u} \quad Cl_u = \frac{Cl}{f_u} \quad Cl_{int} = \frac{Cl_h}{f_u(1 - Cl_h / Q_h)}$$

V_{uss} = unbound volume

Cl_u = unbound Clearance

f_u = fraction unbound in plasma

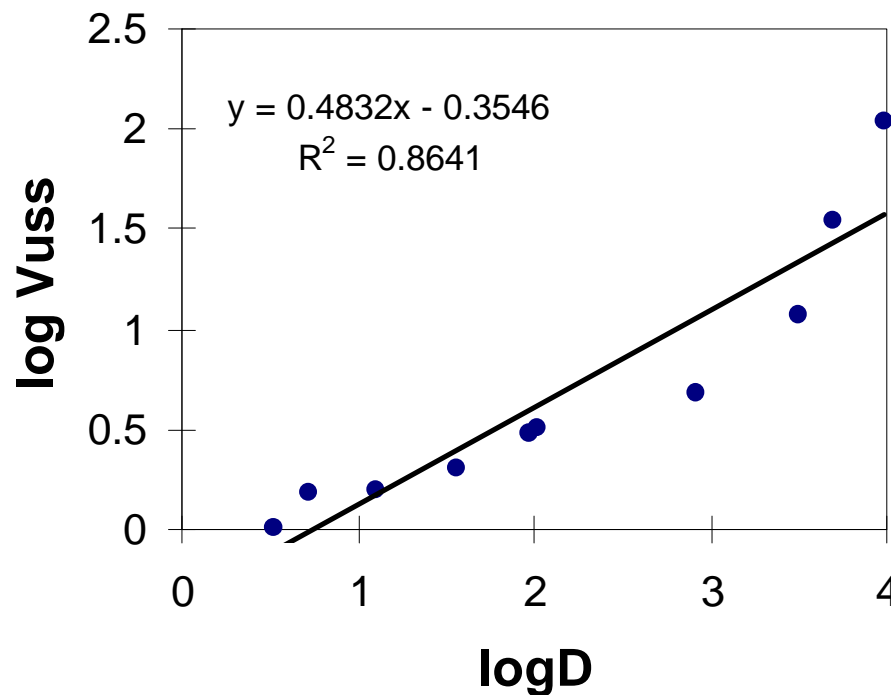
Q_h = hepatic flow

Approach Widely taken

- Cl_h to Cl_{ub} Toon and Rowlands 1979
- V_{ss} to V_{ssu} M Arendt et al 1983
- V_{ssu} x-species M Hiura et al 1984
- K_{pus} PH Hinderling 1988
- physiologic models DA Smith 1988
Bernareggi 1990
Blakey 1997
Ishazaki 1998
Nesterov 1999

Example 1 unbound Vss qsar

- Distribution of 5-ethyl-5-substituted barbituric acids measured in rat

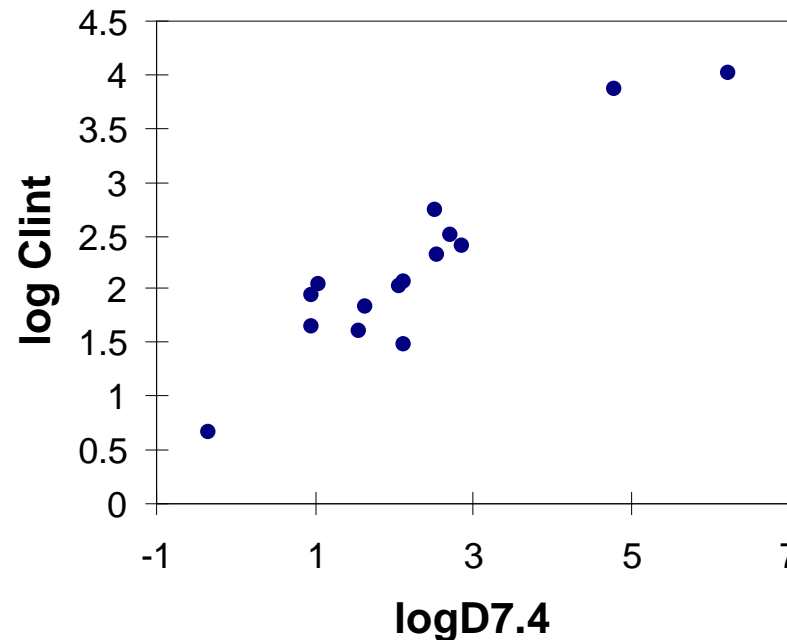


Toon & Rowland (1983): J. Pharmacol. Exp. Ther. 225, 752-763

But of course its implicitly non-linear !!

Example 2 -unbound Cl qsar

- Clearance of diverse CYP-3A4 substrates

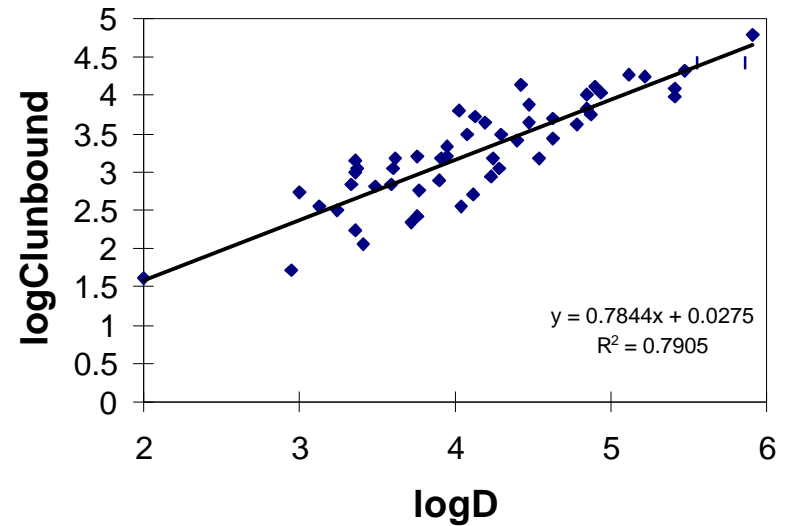
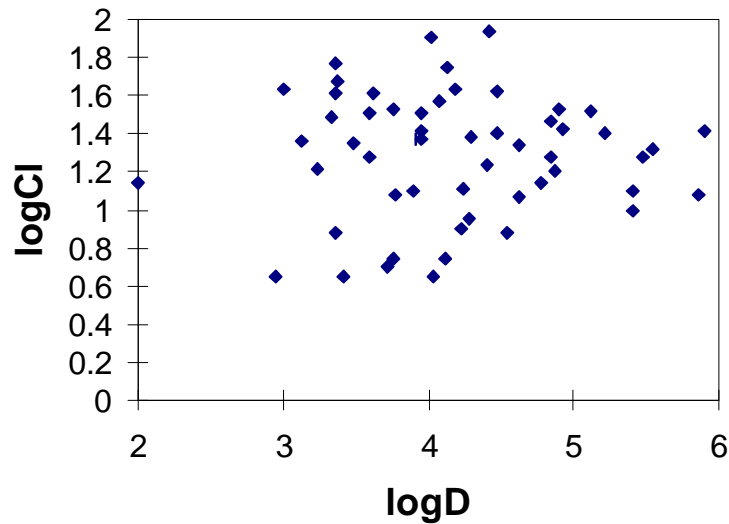


Amiodarone
felodipine
nifedipine
haloperipol
bupivacaine
imipramine
triazolam
diltiazem
alfentanil
quinine
amlodipine
lidocaine
dofetilide
erythromycin
disopyramide

- although different sites of oxidation - same qsar !
- 3A4 has a large open hydrophobic site
- weak hydrophobics and allow motion in the receptor

. Smith DA, Jones BC, Walker DK(1996);, *Med. Res. Rev.*, 16, 243.

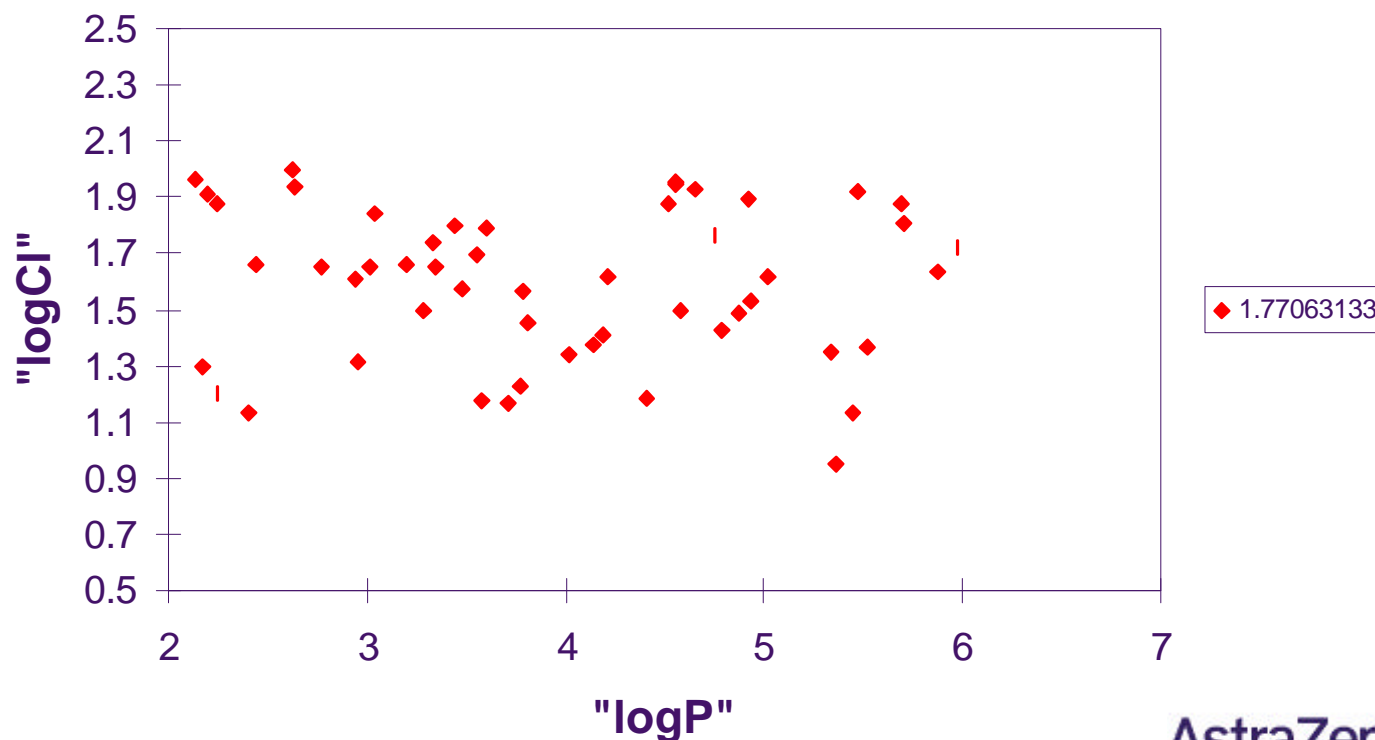
Charnwood Data



Charnwood data

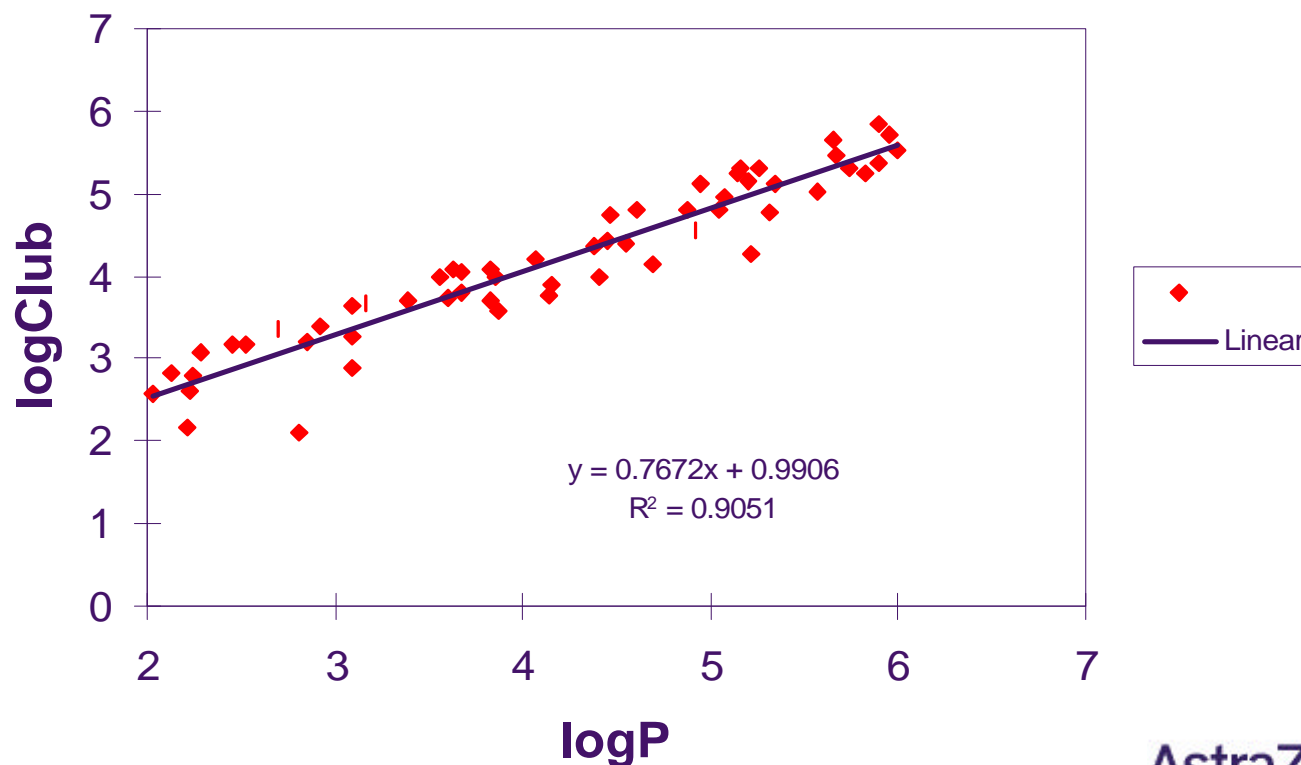
- Check using random nos as CI

Plot of log(random number) vs logP for 58 datapairs

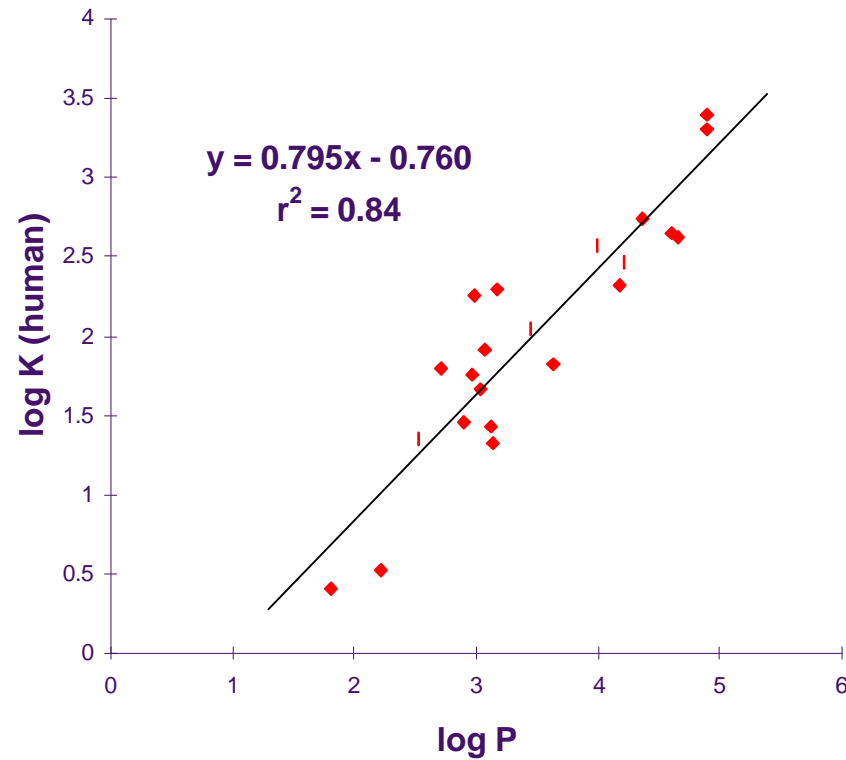


Charnwood Data !

plot of log(Cl-random number)unbound vs "logP for 58 datapairs

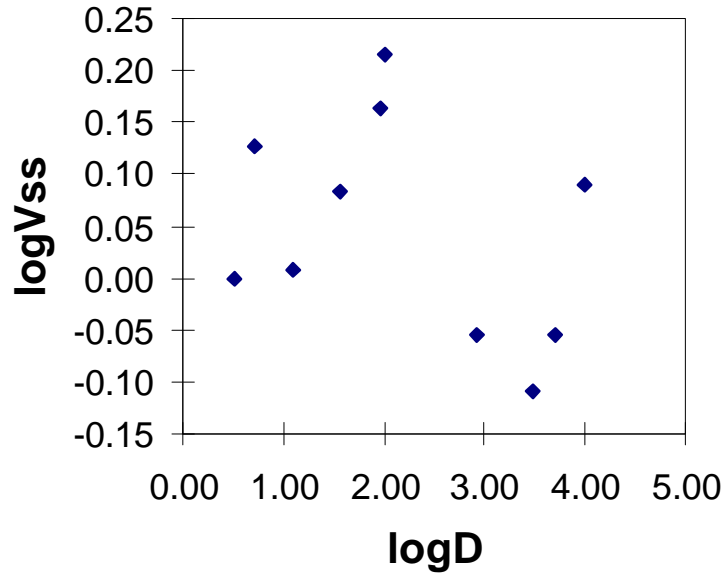


Role of protein binding

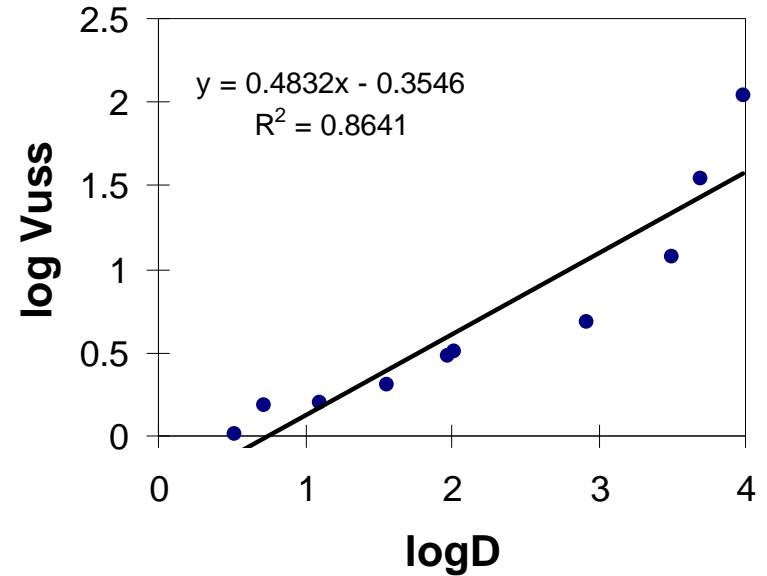


$$\log K = 0.795 \log D - 0.760 \quad \text{where } K = \frac{\% \text{bound}}{\% \text{free}}$$

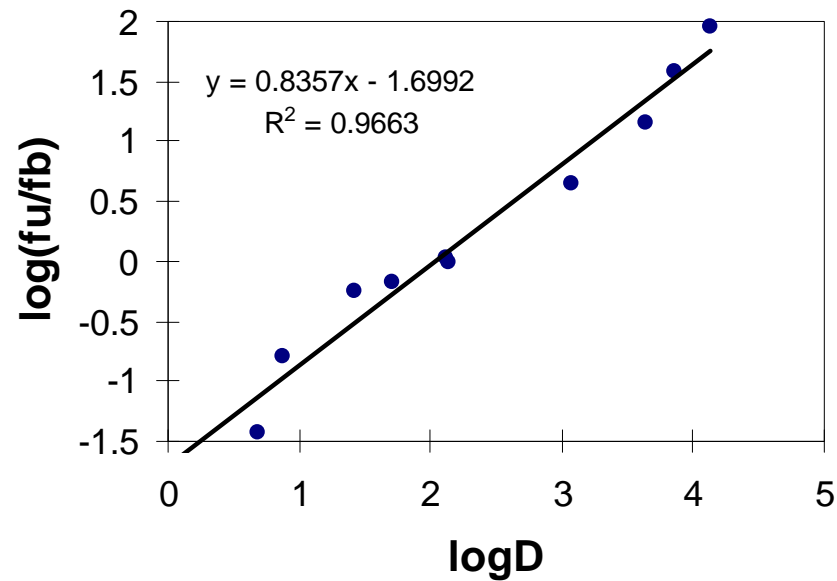
Example 1...



$$V_{uss} = \frac{V_{ss}}{f_u}$$



BUT...



Are there problems

- Correcting to unbound/intrinsic values
 - modifying y with a property already correlated with x
 - often variance $f_u \gg Cl$ or V_{ss}
 - variation in Cl/V_{ss} now minor residual on ppb vs $\log D$
 - example 2 - CYP3A4 substrates
 - Cl range 1-12 ml/min/kg
 - f_u range 0.2 - 0.0002 !!!
 - Randomisation testing
 - a now common approach for significance...

Significance by randomisation

1. Calc observed correlation

logD	CI	fu	Club
1	2	.1	20
2	10	.01	1000
3	3	.001	3000
4	15	.0001	15000

$$\log\text{Club} = M \cdot \log D + \text{const} \quad r^2 = \dots$$

2. Randomise CI,
recalc logClub vs logD correlation

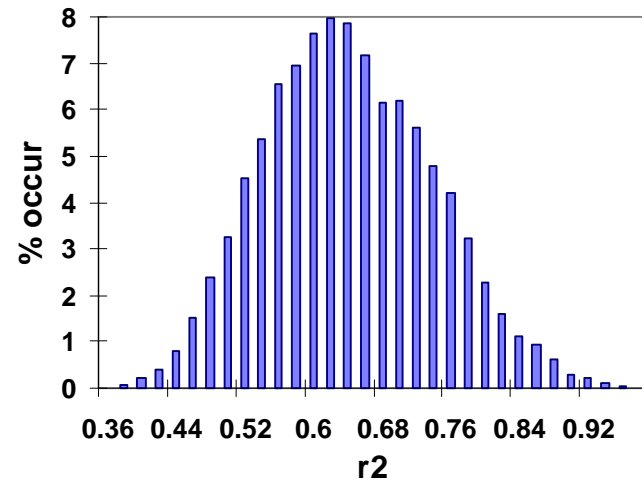
logD	CI	fu	Club
1	3	.1	30
2	15	.01	1500
3	10	.001	10000
4	2	.0001	20000

$$\log\text{Club} = M \cdot \log D + \text{const} \quad r^2 = \dots$$

3. Repeat 10,000 times

logD	CI	fu	Club
1	15	.1	20
2	3	.01	1000
3	2	.001	3000
4	10	.0001	15000

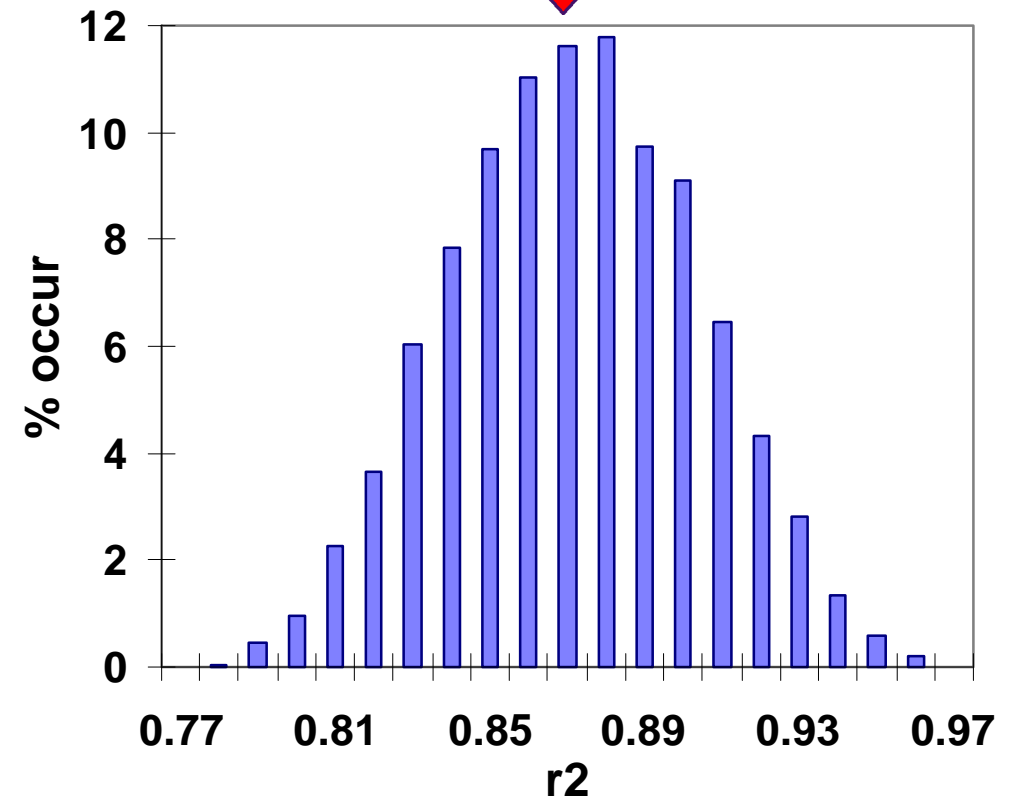
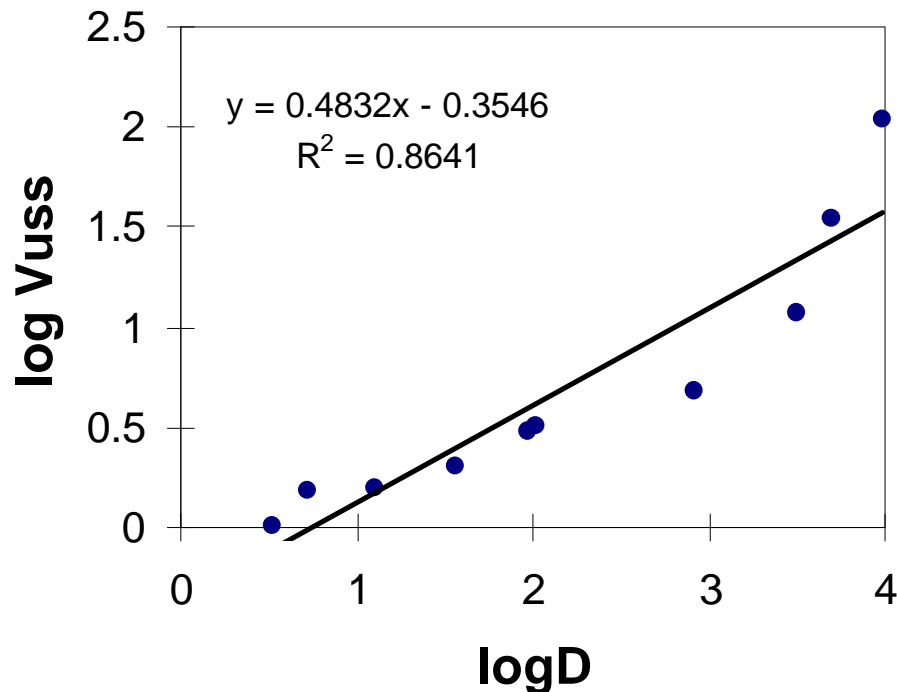
$$\log\text{Club} = M \cdot \log D + \text{const} \quad r^2 = \dots$$



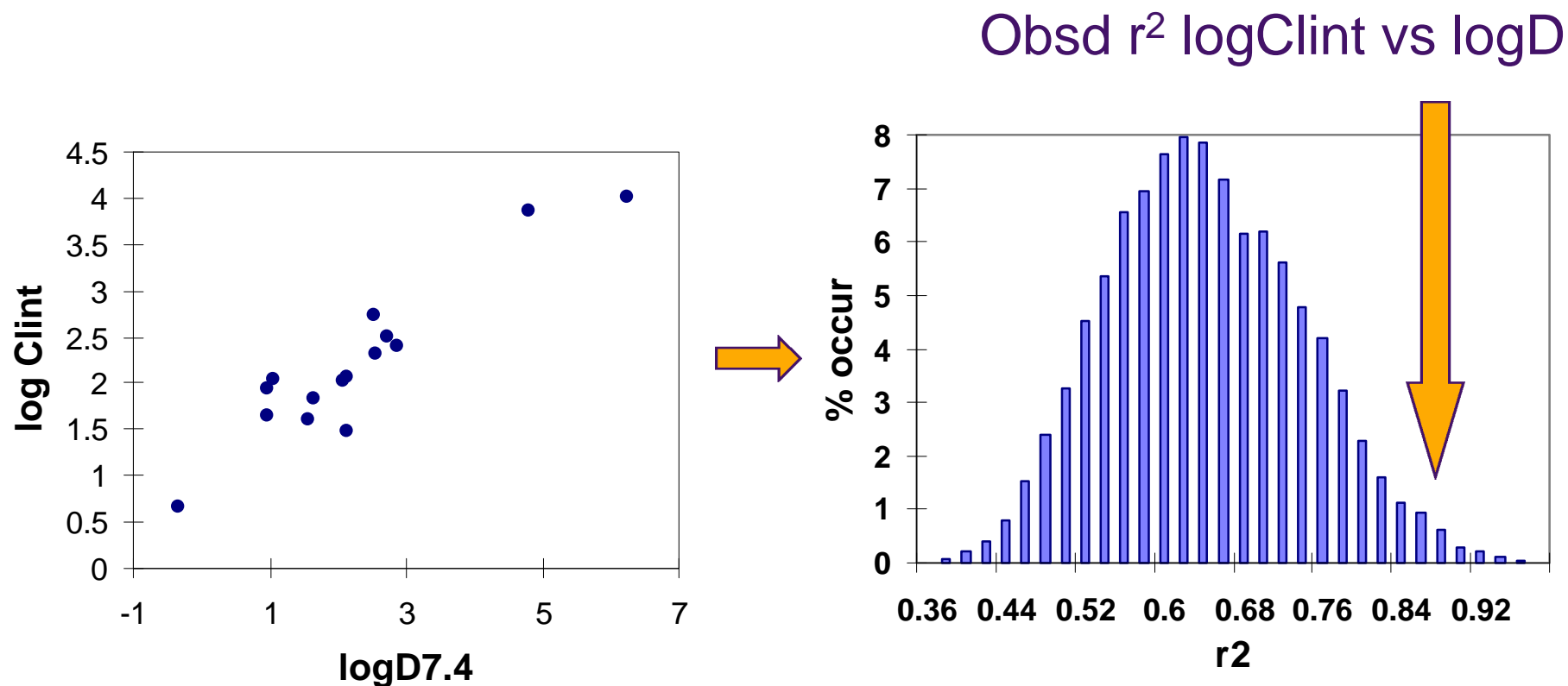
Example 1

Distribution of 5-ethyl-5-substituted barbituric acids measured in rat

Obsd r2 logVuss vs logD



CI of diverse 3A4 substrates



- Correlation significant $p = 0.012$!

Smith DA, Jones BC, Walker DK(1996):, *Med. Res. Rev.*, 16, 243.

Statistical Modelling (Dave Salt)

$$Y = W + kX$$

Y = transformed var

W = untransformed var

X = logD

$$r_{xy} = \frac{l r_{xw} + k}{\sqrt{l^2 + k^2 + 2klr_{xw}}}$$

ρ_{xy} = correlation xy ρ_{xw} = correlation xw
 $l^2 = \sigma_w^2 / \sigma_x^2$ $k = \text{const}$

example 1

$$\log V_{uss} = \log V_{ss} - \log fu$$

$$l^2 = \sigma_{V_{ss}}^2 / \sigma_{\log fu}^2$$

$$k = -1$$

$$\rho_{xw} = \rho_{V_{ss}, \log fu} = -0.356$$

$$\rho_{xy} = 0.9779$$

$$\text{obsd } \rho_{xy} = 0.9781 !!$$

Statistical Modelling 2

$$Y = W + kX$$

Y = transformed var

W = untransformed var

X = logD

$$r_{xy} = \frac{l r_{xw} + k}{\sqrt{l^2 + k^2 + 2kl r_{xw}}}$$

ρ_{xy} = correlation xy ρ_{xw} = correlation xw
 $l^2 = \sigma_w^2 / \sigma_x^2$ $k = \text{const}$

example 1

$$\log V_{uss} = \log V_{ss} - \log fu$$

$$\log fu = 0.467 - 0.513 \log D$$

$$\log V_{uss} = \log V_{ss} + 0.513 \log D$$

$$K = 0.513$$

$$l^2 = \sigma_{V_{ss}}^2 / \sigma_{\log D}^2 = 0.083$$

$$\rho_{xw} = \rho_{V_{ss}, \log D} = -0.356$$

$$\rho_{xy} = 0.9872$$

$$\text{obsd } \rho_{xy} = 0.929$$

Example 1...

If x and w are uncorrelated, i.e. $\rho_{xw} = 0$

$$r_{xy} = \frac{k}{\sqrt{l^2 + k^2}}$$

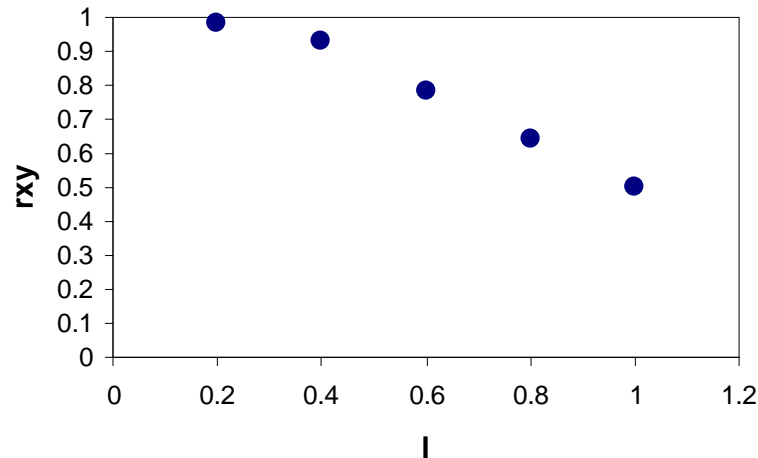
X, Y can still be correlated depend on k/l

Fix $k = 0.5$ (*as in the real example*)

change l 1, 0.8, 0.6, 0.4, 0.2, 0.1 (*0.29 real e.g.*)

$k = 0.5, l = 0.2 - 1$

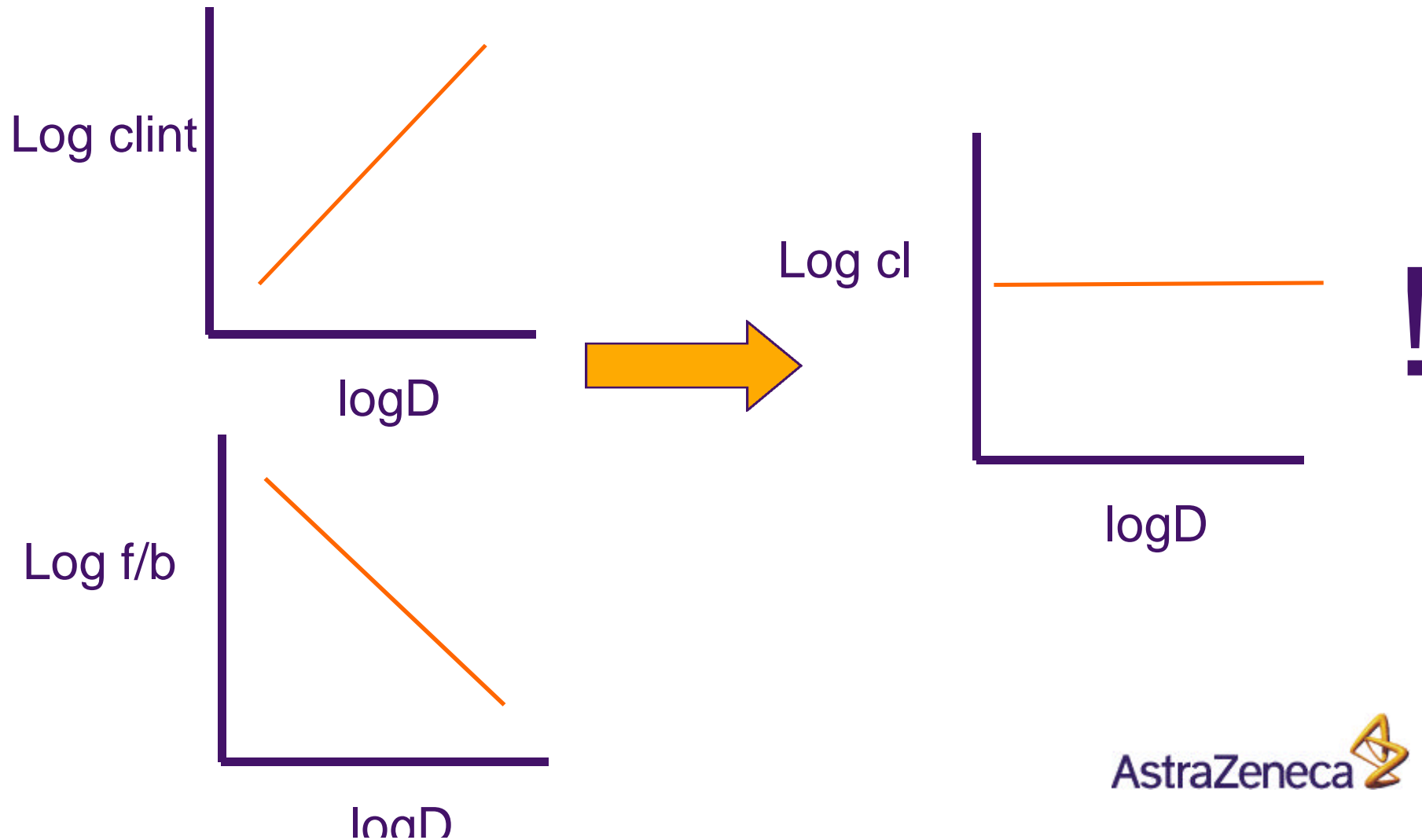
$$l^2 = \sigma_w^2 / \sigma_x^2$$



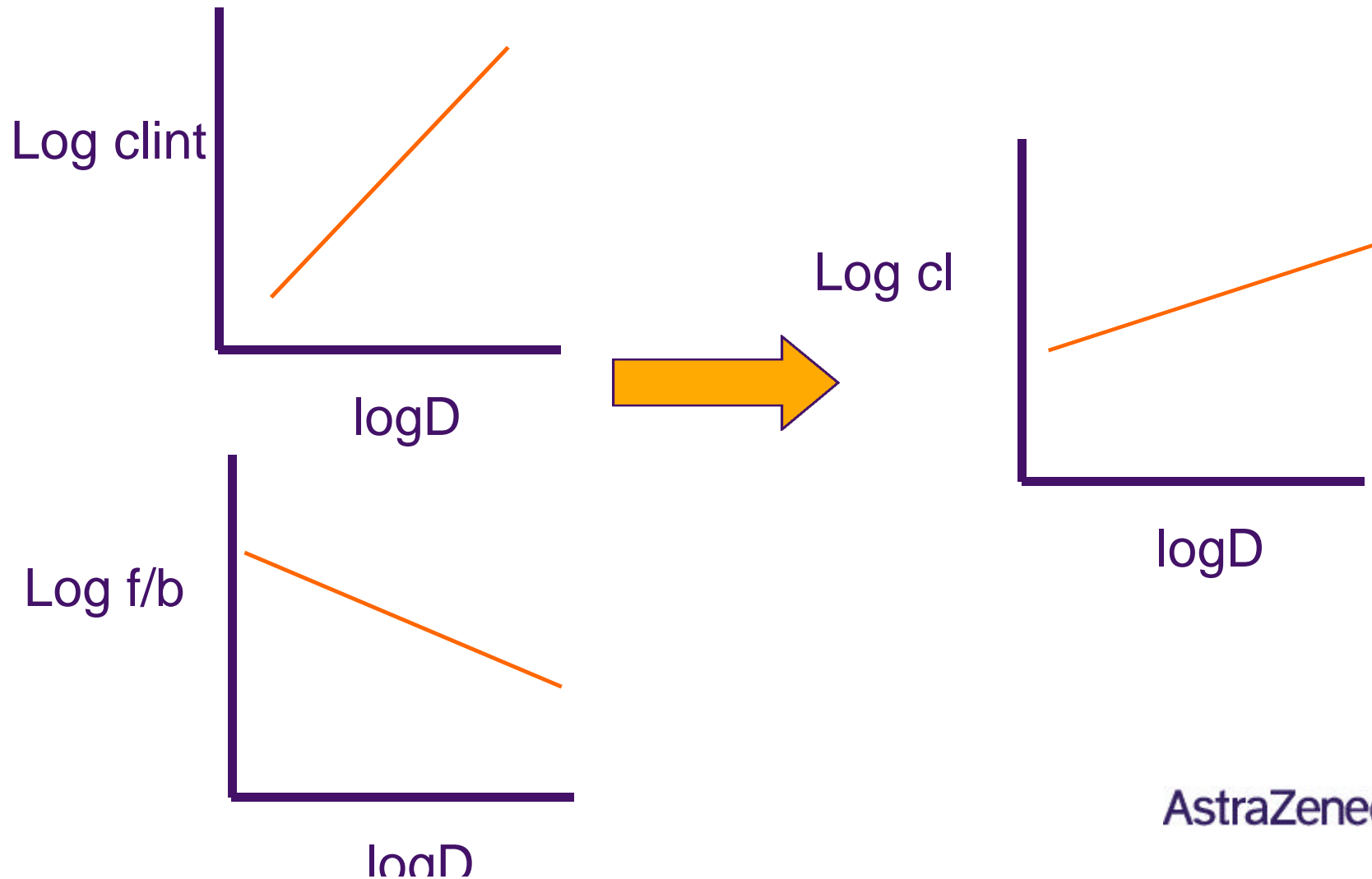
Does correcting to unbound help ?

- Correcting Cl/V_{ss} for ppb -
 - statistical ambiguity introduced
 - randomisation would protect from spurious correlation
- unbound values estimate intrinsic Cl/V_{ss}
 - hepatocyte/microsome assay - Cl_{int} directly
 - what if Cl_{int} itself really is related to $\log D$?

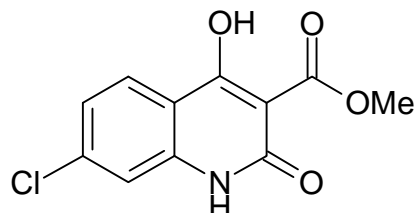
If Cl_{int} really correlated to $\log D$



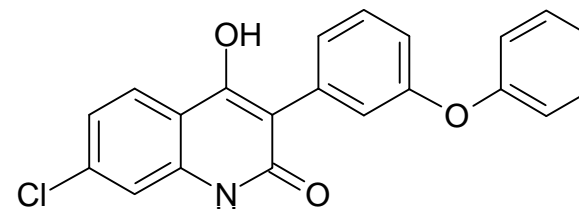
If Cl_{int} really correlated to $\log D$



Glycine/NMDA Antagonists

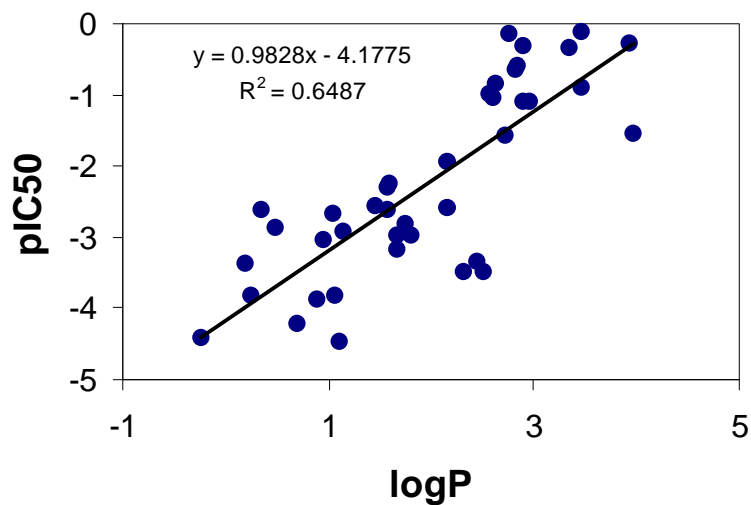


IC₅₀ = 6.5 μ M
ED₅₀ = 52 μ mol/kg

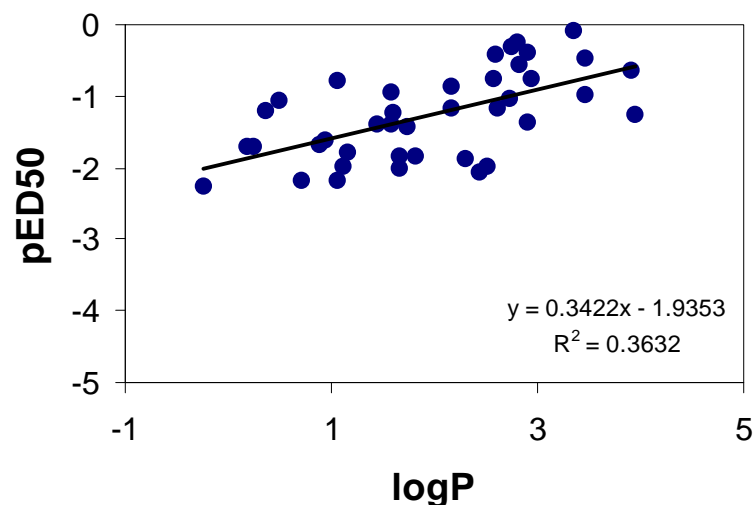


IC₅₀ = 2.2 nM
2.6 μ mol/kg !!

in-vitro pIC₅₀



in-vivo pED₅₀

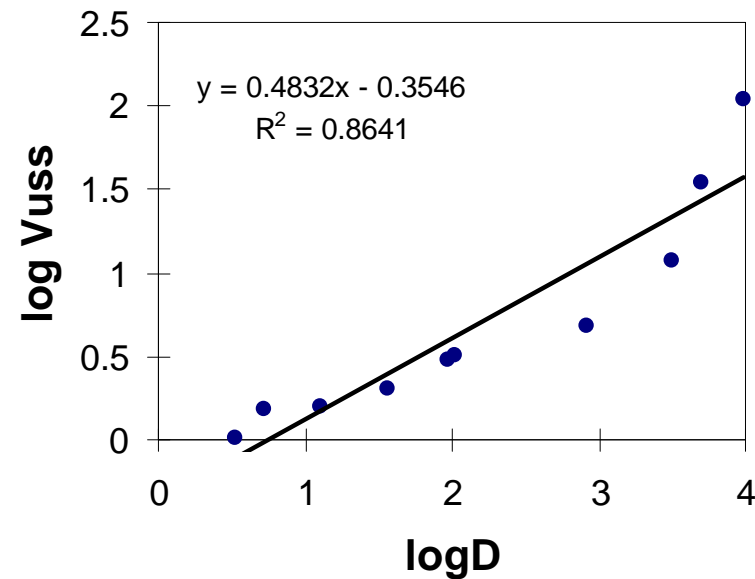
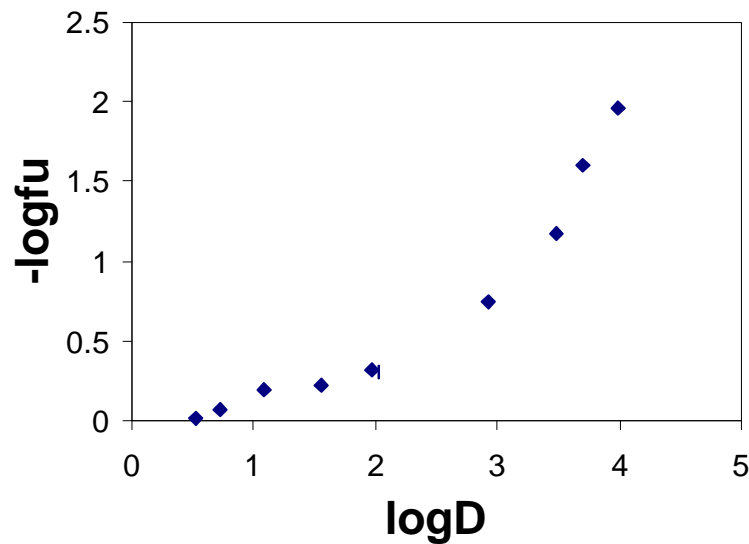


$$pED_{50} = 0.4pIC_{50} + 2.02$$

Rowley et al J. Med. Chem 1997, 40, 4053

Example 1 unbound Vss qsar

- Distribution of 5-ethyl-5-substituted barbituric acids measured in rat



Toon & Rowland (1983): J. Pharmacol. Exp. Ther. 225, 752-763

Summary

- Unbound correlations with logD common !
 - unbound QSARS
 - basis of physiologic model tissue k_{pu}s
 - X-species correlations !
- Danger of spurious correlation high
 - depends largely upon ratio σ^2 's x,w- usually high
 - true significance can be assessed by randomisation
- The problem is simple
 - the maths are not so simple
 - modelling in progress