

Intravenous Anesthetics – studies from microsomes to big animals!

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ISAP 2014

1970s – Why study intravenous anesthesia?

- Toxicity of volatile agents

- But no ideal IV drug:

THIOPENTAL (and other barbiturates)

Propanidid – withdrawn 1984

Ketamine

Althesin 1971 – withdrawn as Cremophor formulation, 1984

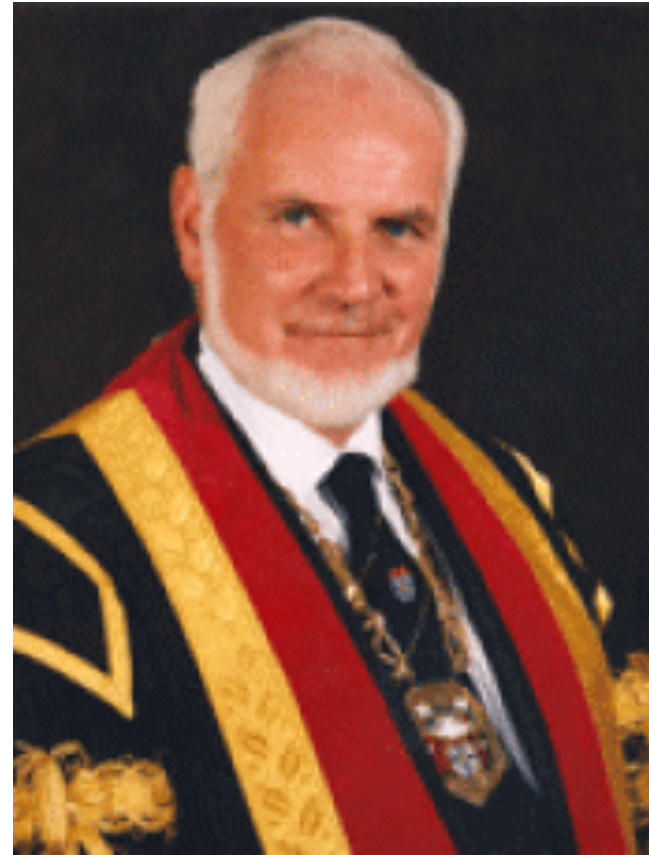
Etomidate 1971

Adverse effects of barbiturates: - either avoid their use or give in reduced dosage (1974).

- Hx of porphyria
- Hx of allergy; Hx of severe asthma
- Hx of epilepsy (for methohexital)
- Hypovolemia – CVS depression. Potential for laryngospasm
- Not stable in solution; high alkalinity; vascular toxicity
- Antanalgesic in small doses
- Severe uremia
- Severe cardiac disease (IHD; malignant or UT HT)
- Prolonged recovery (and abn. LFTs) after large doses or continuous infusions – altered kinetics (drug cumulation) and active metabolite.

Once upon a time!

Two teachers!

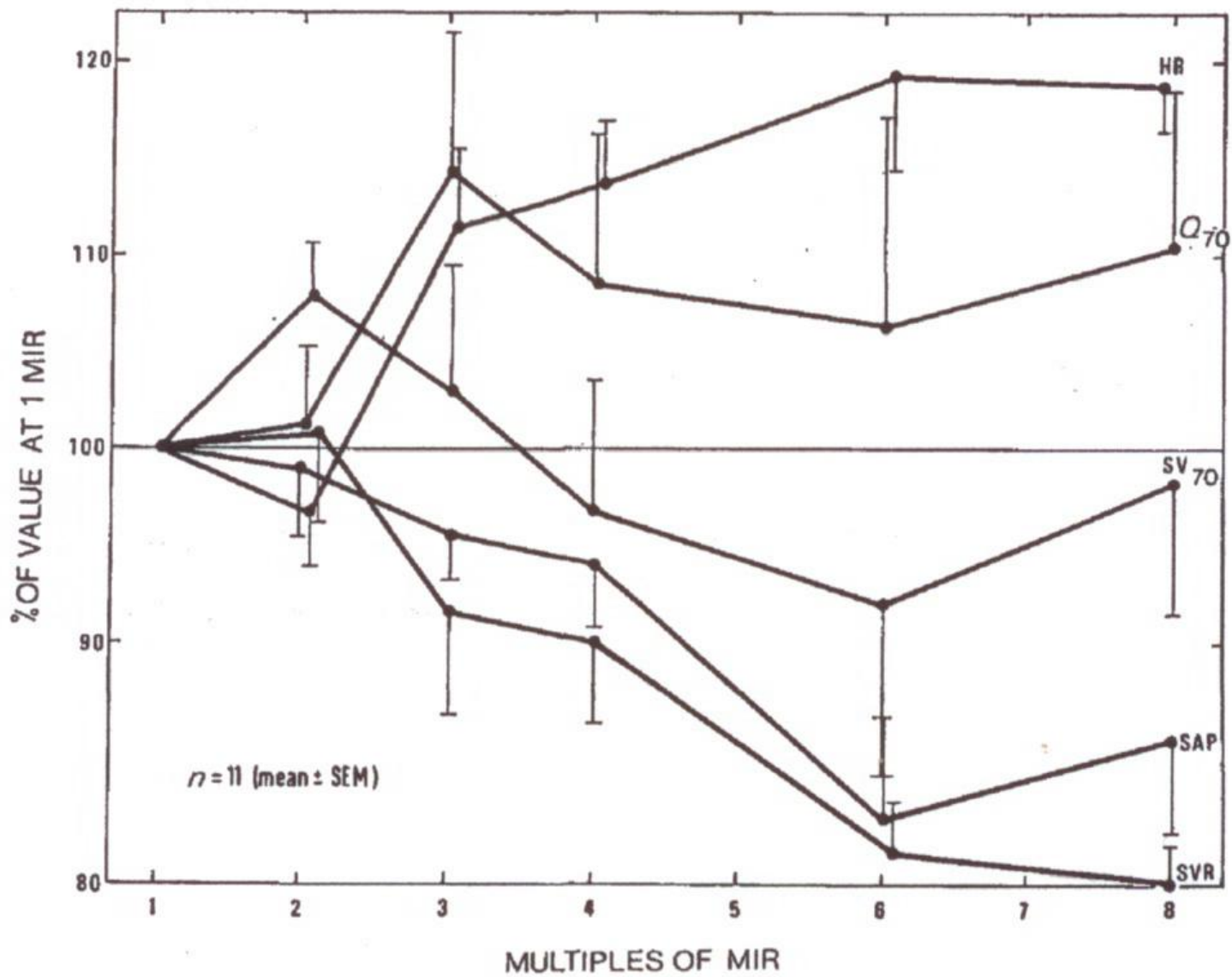


Questions – 37 years on! What are some of the key questions?

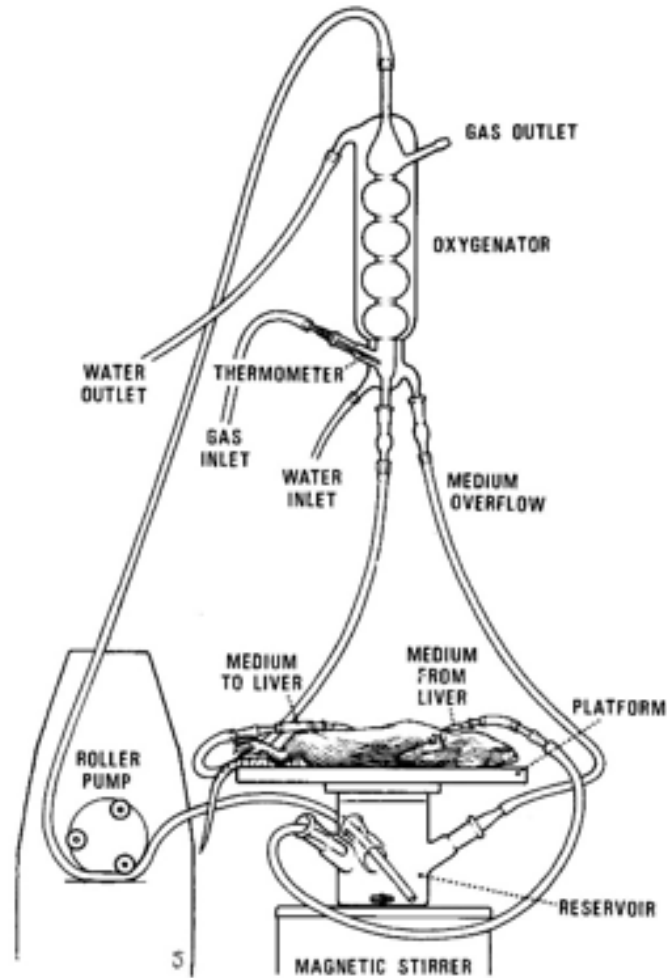
- Evaluation of new drugs – are they better than existing ones?
- Drug interactions – do they affect kinetics or dynamics?
- Mechanisms of anesthesia – diverse structures; why similar? Can we design drugs with no side-effects?
- Application of IV anesthesia to animal species – does allometry work? What end-points to assess efficacy?
- Dosing approaches for large animals – are side-effects predictable?

Types of experimental model:

- In vitro studies: microsomes and hepatocytes, and isolated perfused rat liver
- In vivo studies: rat; man; and other animal species
- Studies in large animals – horses; camels; ‘large cats’
- Use of computational chemistry

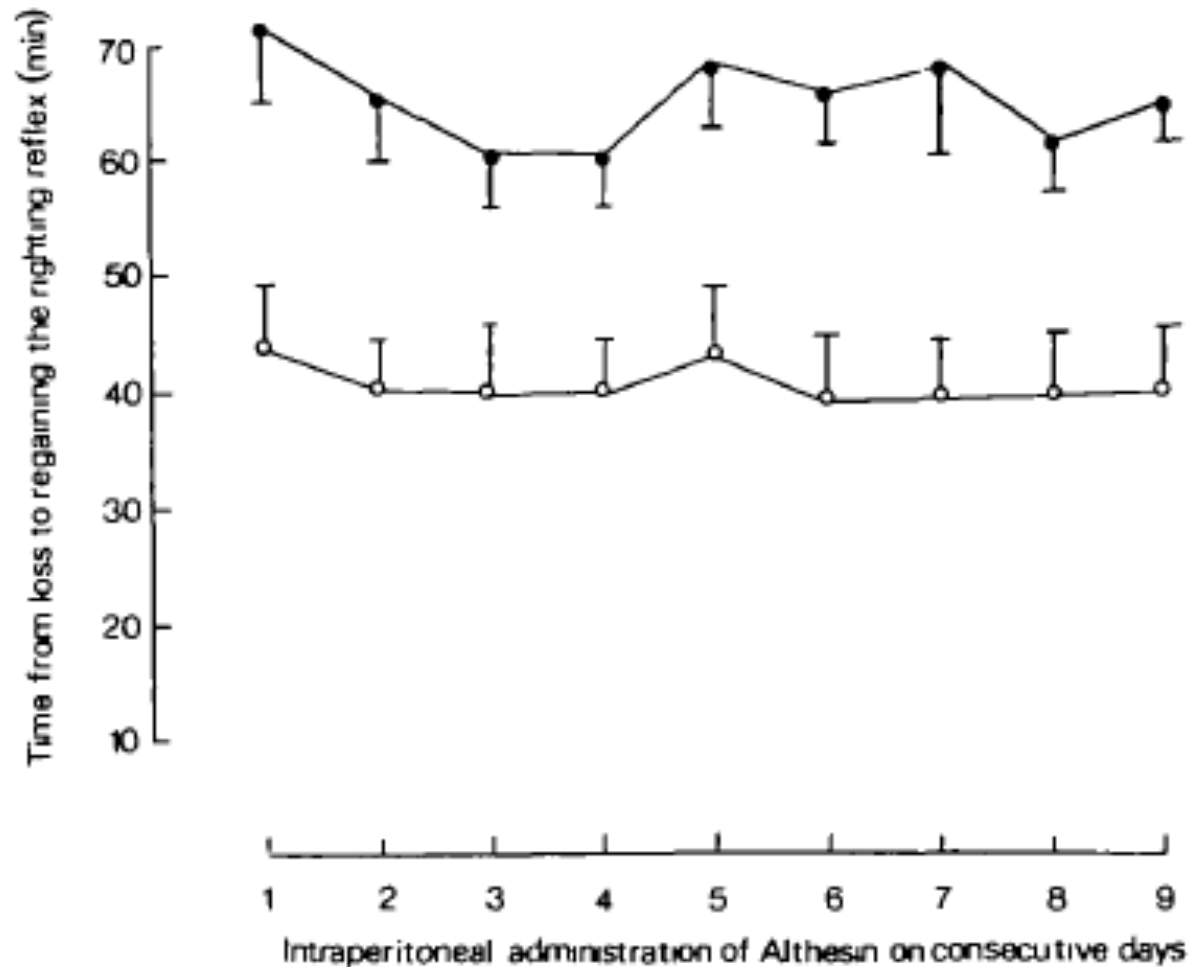


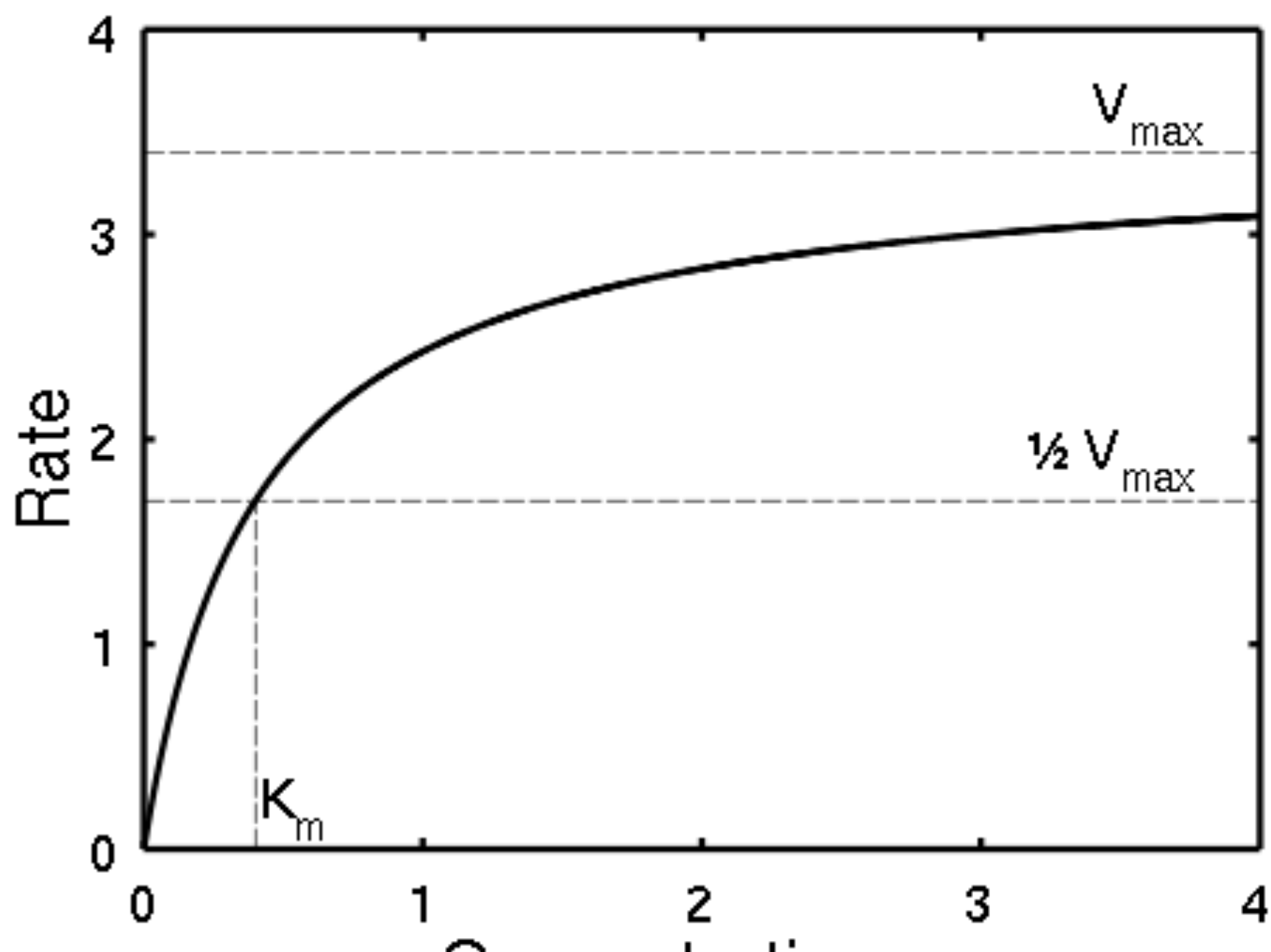
But does alphaxalone accumulate when given in large doses?



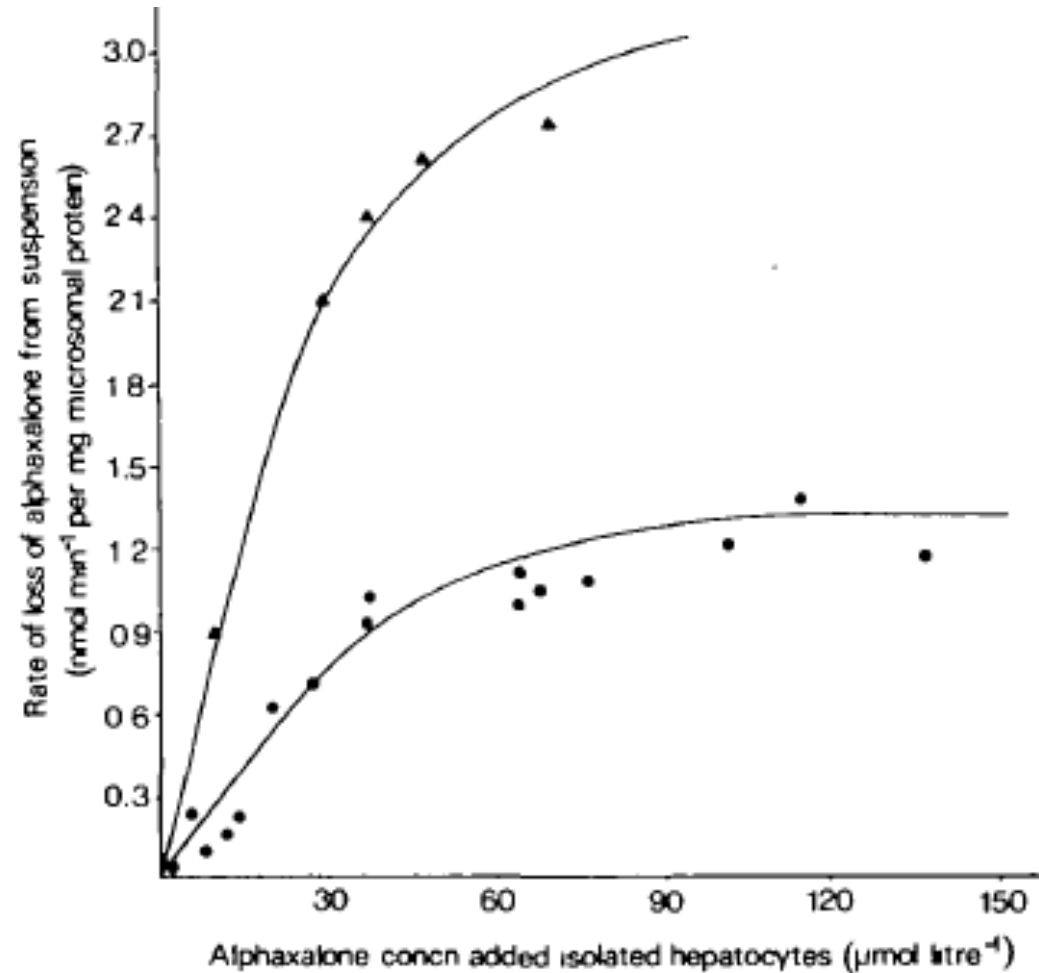
APPARATUS FOR PREPARATION OF ISOLATED RAT HEPATOCYTES

Q1: Does alphaxalone induce its own metabolism (cf ketamine)?

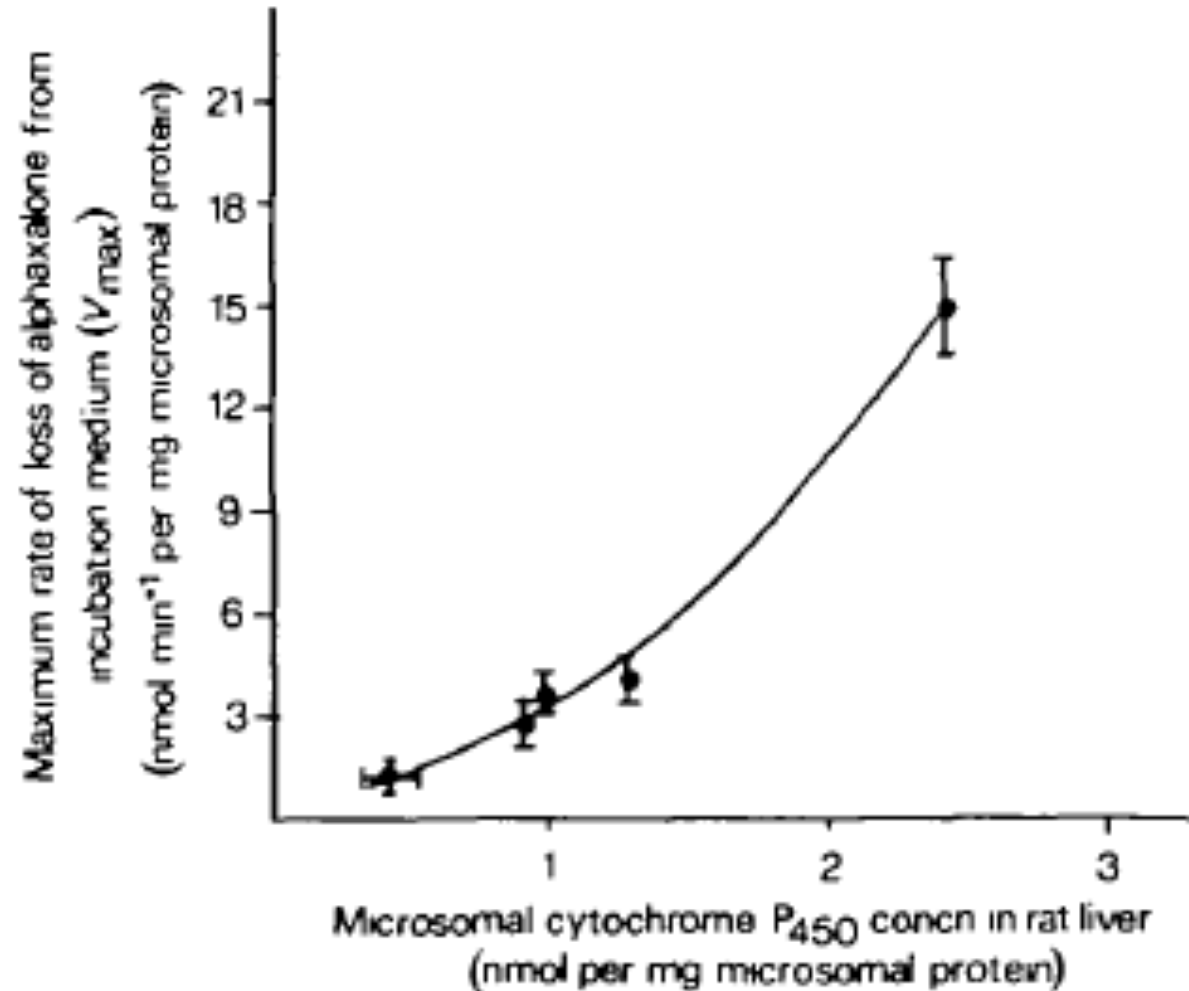




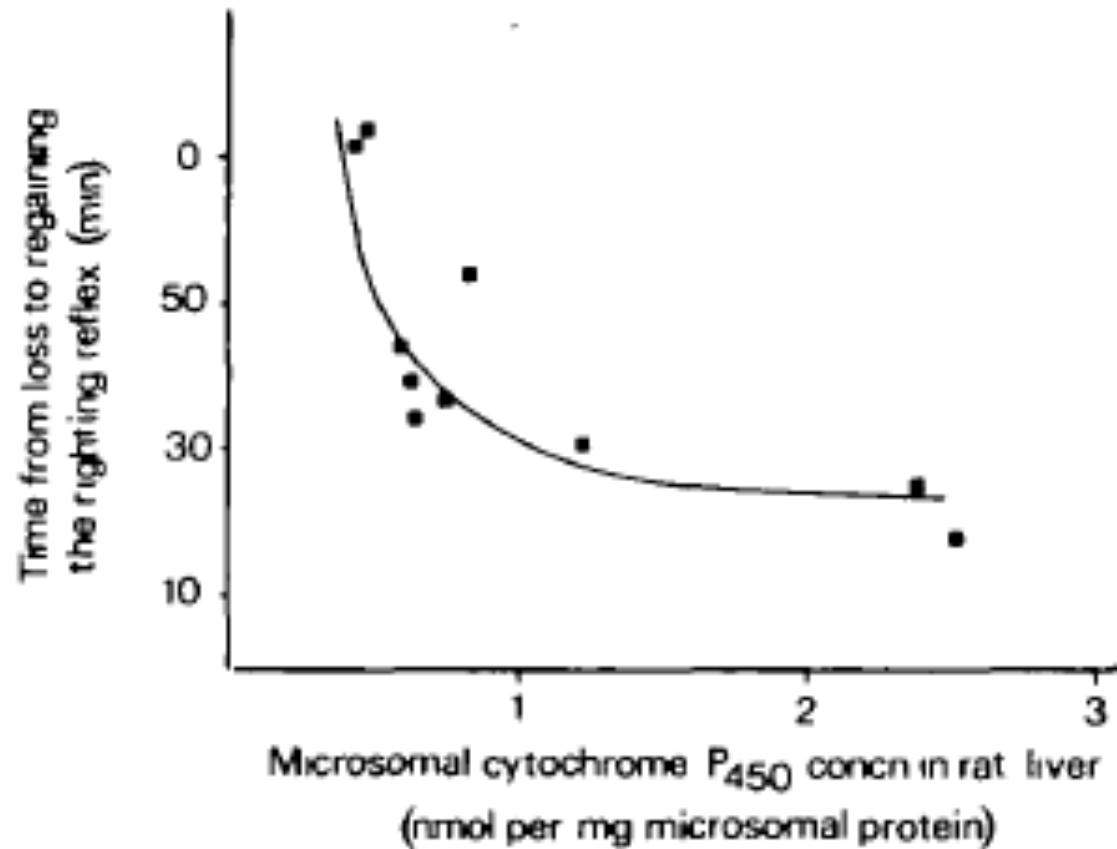
Q2: What are the kinetics of alphaxalone metabolism?



Q3: Alphaxalone metabolism – role of CYP 450



Q4: CYP 450 and recovery from anesthesia



Comparative rates of alphaxalone clearance:

Microsomes (female): 0.031 (0.009) ml/min/nmol P450

Hepatocytes (male): 0.27 (0.035) "

Man in vivo:
(male and female) 0.123 (0.022) "

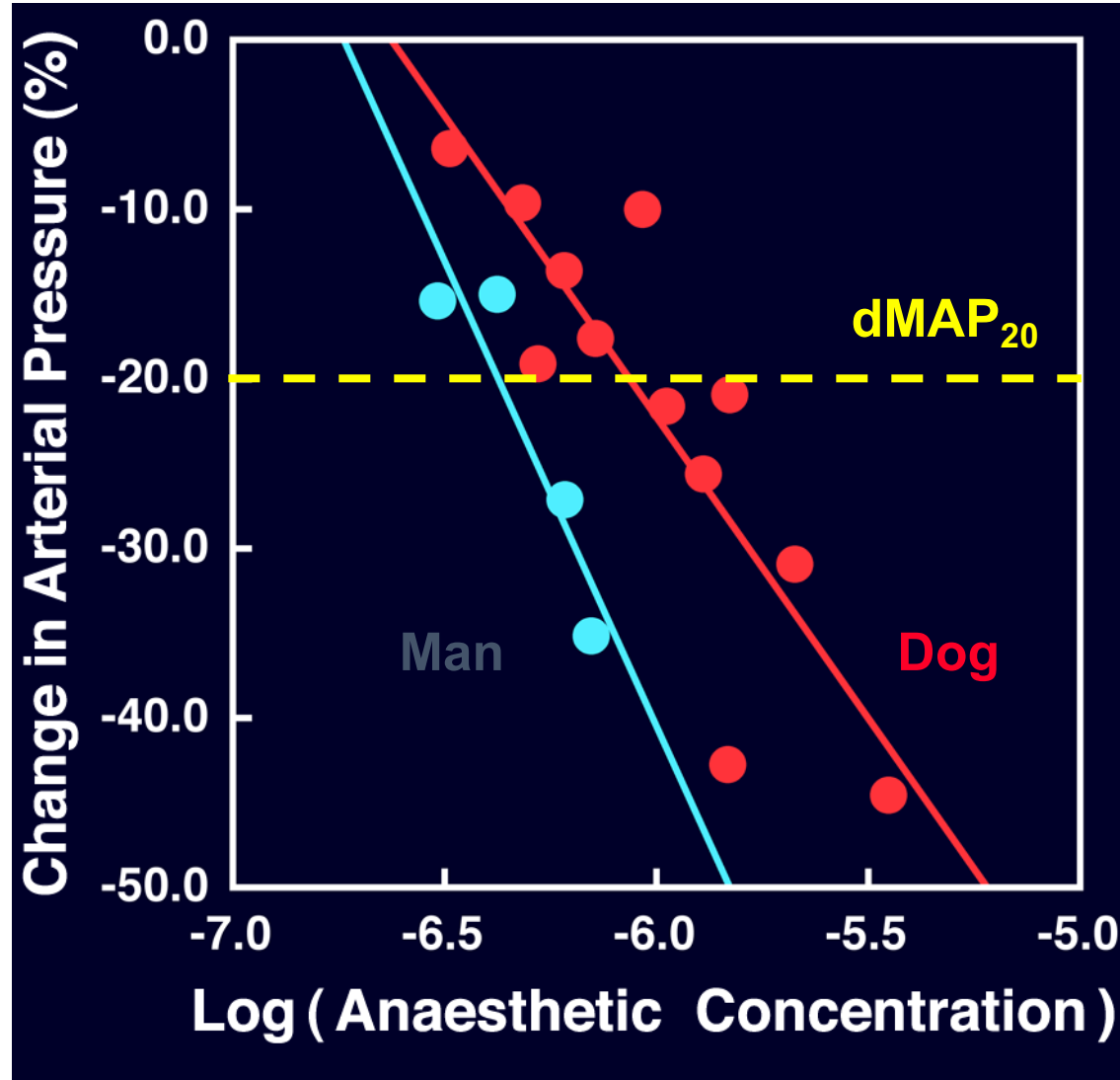
Then came propofol (1983)!

Why explore for further new drugs? What are the main adverse effects of propofol?

- Dose-related cardio-respiratory depression
- Lipid toxicity after prolonged infusions (PRIS)
- Pain on injection

- Lipid formulation – risk of contamination and bacterial growth
- Incompatible with plastic containers (especially with RSL)

Depression of MAP: Propofol



Data from Dogs:

Puttick et al

Piriou et al

Nakaigawa et al

Data from Man:

Le Page et al

Larsen et al

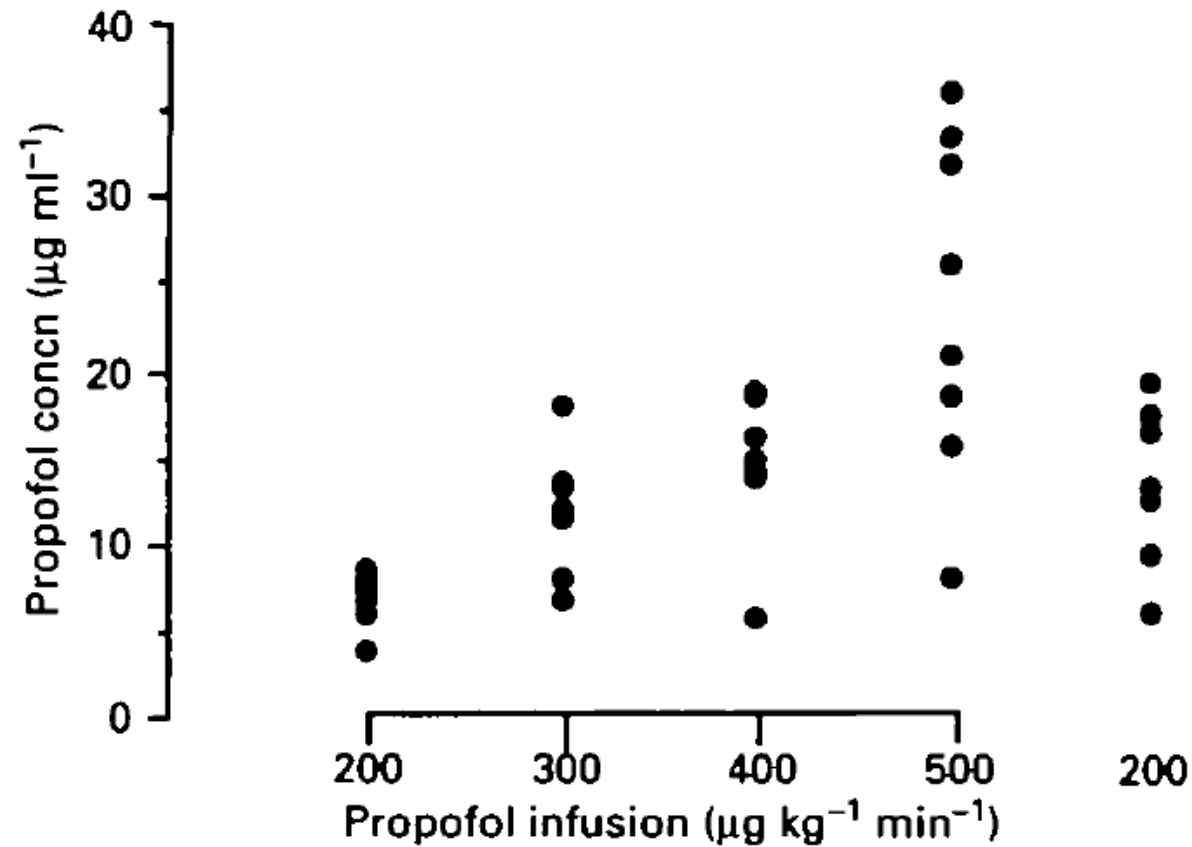
Coates et al.

Effect of drug concentration on propofol clearance

Are the kinetics linear? (infusion rates: 25-200 $\mu\text{g}/\text{kg}/\text{min}$)

- Sear et al, 1994 (decreased LBF – dog)
- Coetzee et al, 1995
- Schnider et al, 1998
- Kurita et al, 2002

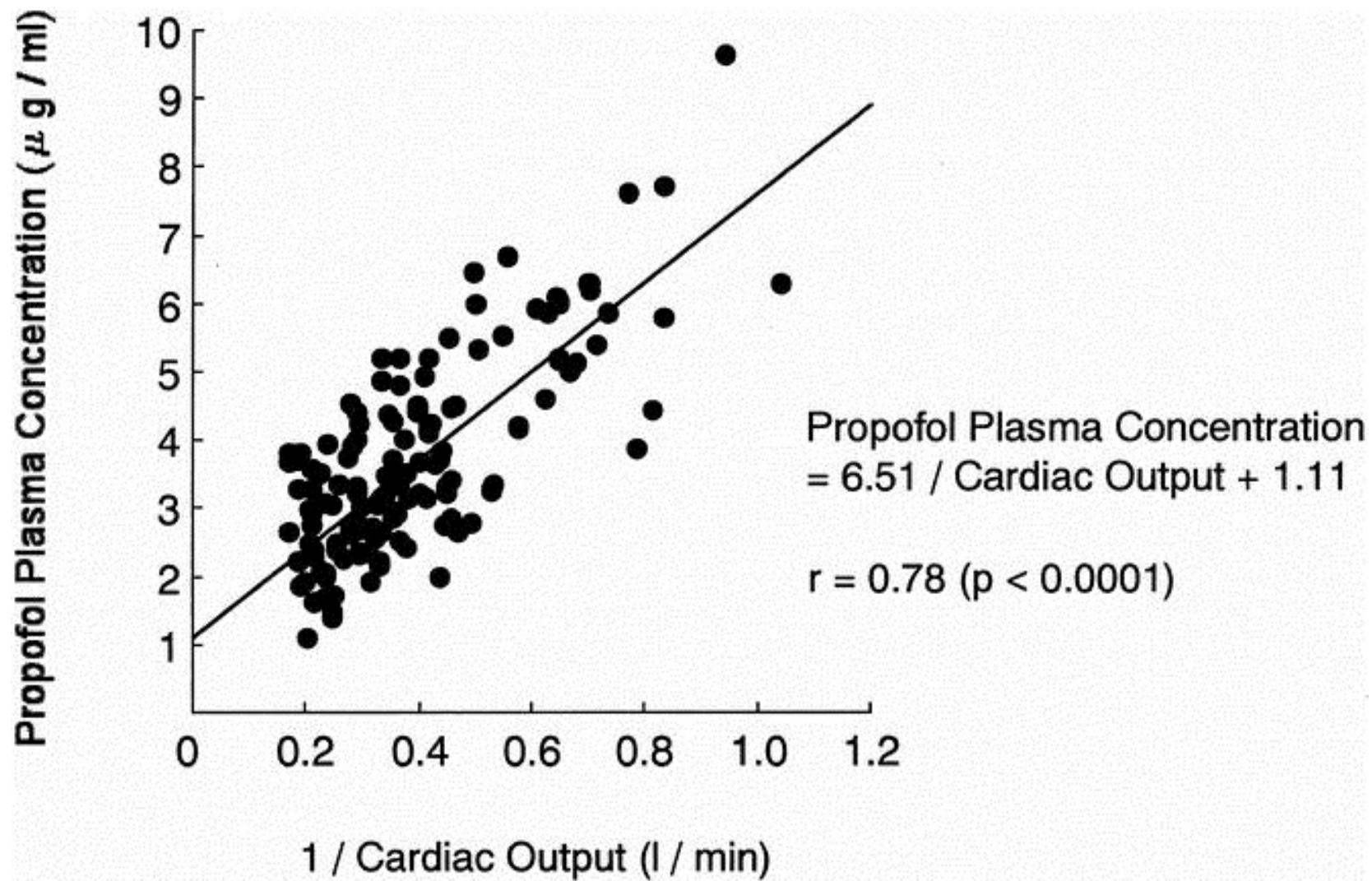
J W SEAR, J Diedericks, P Foex (1994) Continuous infusions of propofol administered to dogs: effects on ICG and propofol disposition. British Journal of Anaesthesia 72; 451-455



Effect of propofol concentration on cardiac output and ICG clearance

Infusion Rate ($\mu\text{g}/\text{kg}/\text{min}$)	Prop C ($\mu\text{g}/\text{mL}$)	BP	Q (mL/min)	ICG Clp (mL/min)
200	7.03	120/88	1605	154.1
300	11.97	109/78	1432*	115.8*
400	14.64	95/67	1355*	97.2*
500	23.85	81/51	1135*	88.2**

Sear et al, BJA 1994



Can we reduce the side-effects of iv. anesthetics:

Especially cardiovascular depression?

The future?

Can we use computational drug modeling to minimise the cardio-depressive effect of propofol, and develop a new rapid onset and offset hypnotic agent?

Cardiovascular CoMFA for 12 IV Agents.

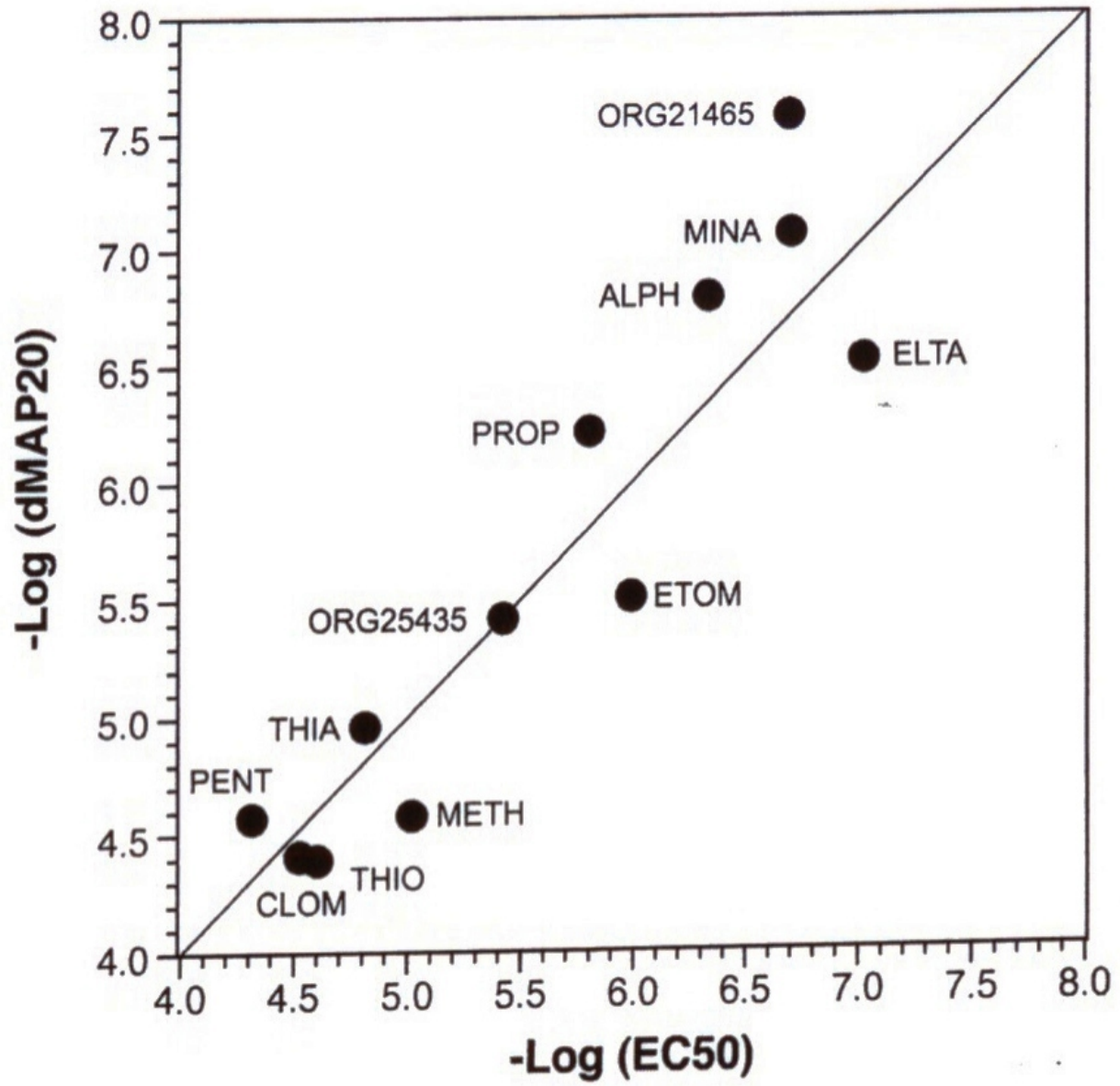
Steroidal Anaesthetics (n = 4): alfaxalone, eltanolone, minaxolone, ORG 21465

Barbiturates (n = 4): Thiopental, methohexital, pentobarbital, thiamylal.

Miscellaneous (n = 4): Etomidate, propofol, clomethiazole, ORG 25435

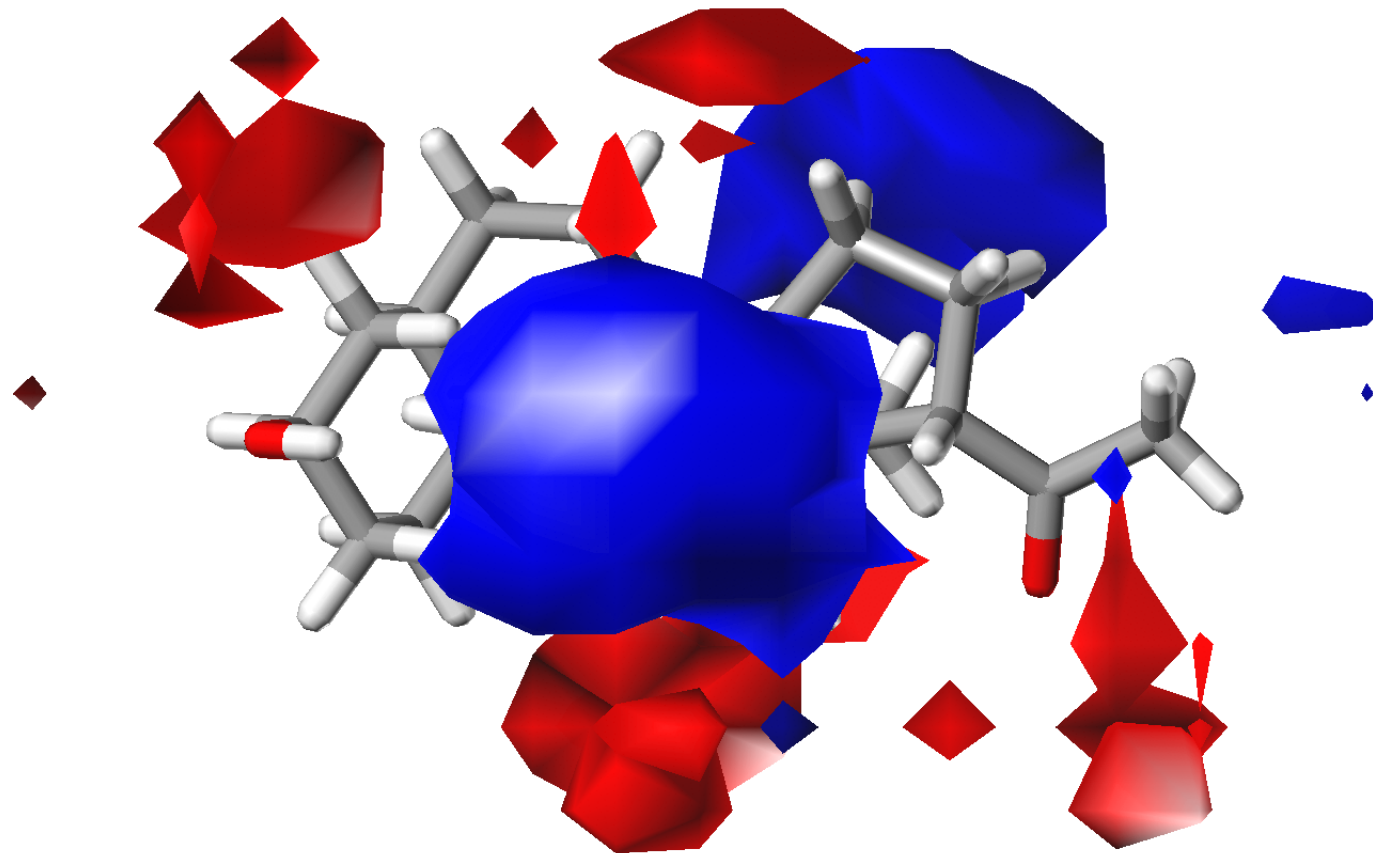
Modelling approach:

- The same lead compound was used for both models – immobilization and cardiovascular depression (eltanolone)
- CoMFA models derived using SYBYL v7.3 (Tripos Inc)
- Low-energy conformers aligned by field-fit minimization technique



A Pharmacophore: (IUPAC, 1998) –
‘an ensemble of steric and electronic features that is necessary to ensure the optimal supra-molecular interactions with a specific biological target and to trigger (or block) its biological response’.

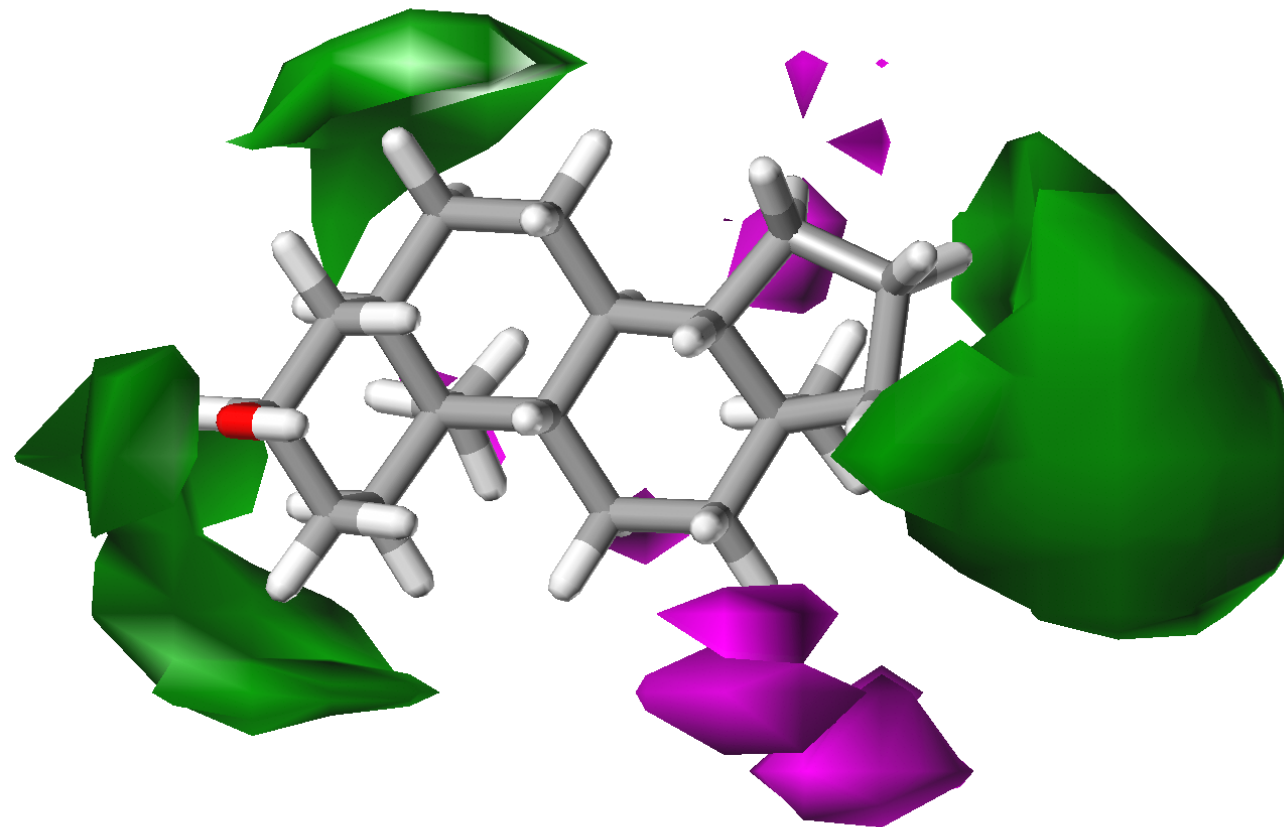
Electrostatic Regions Favoured for Cardiovascular Depression (dMAP20)



■ -ve Favoured ■ +ve Favoured

2Å

Steric Regions Favoured for Cardiovascular Depression (dMAP20)



■ Bulk Favoured ■ Bulk Disfavoured

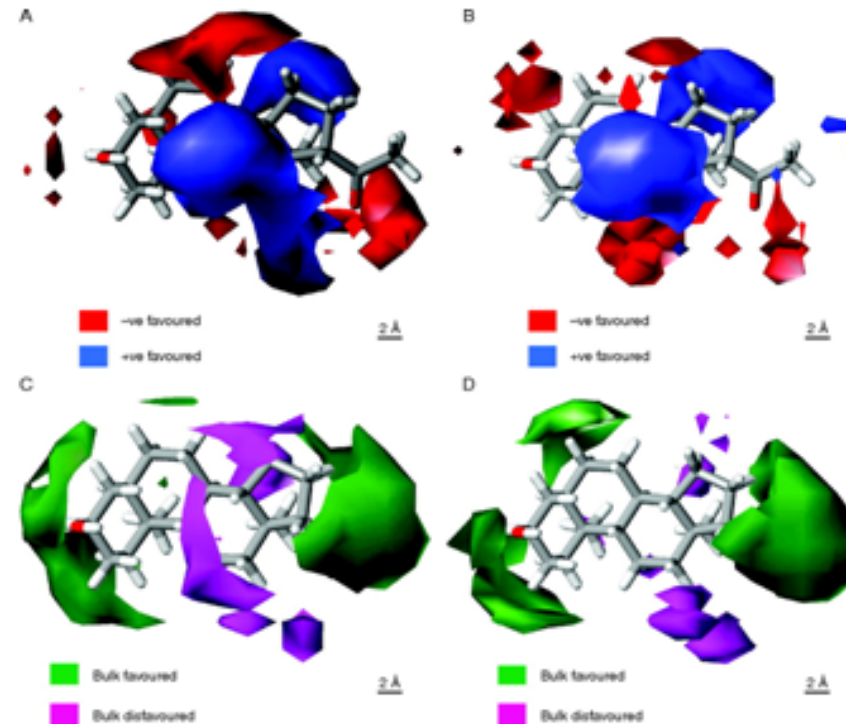
2Å

Comparison of the fit of the CoMFA models

<u>Model</u>	<u>LV</u>	<u>r2</u>	<u>q2</u>	<u>F</u>
dMAP20	1	0.96	0.839	239.638
Potency	2	0.987	0.823	343.66

'Anaesthesia' and cardiovascular depression – can we separate them?

Electrostatic (A and B) and steric pharmacophores (C and D) for plasma free drug concentrations and associated immobilizing activity in 50% patients or animals [EC₅₀] and 20% depression of MAP during continuous infusion of anaesthesia (dMAP₂₀)



Comparison of pharmacophore maps

When isocontours are constructed linking points with the greatest 40% of values, there is a high degree of similarity between the two pharmacophores:

- 87.6% of the electrostatic key regions
- 86.2% of the steric key regions

Conclusions:

These data indicate a substantial commonality in the molecular bases for cardiovascular depression and immobilising activity of iv. agents; and that development of new agents without cardiovascular depressive effects may be a challenge.

Harper's Crystal - aka 'Spike'



1990s - Request for help from Two Veterinary Anaesthetists at 'The Other University'!

Dr Leslie Hall



Dr Leslie W Hall (1927-2010)

Dr Polly Taylor



Intravenous anesthesia in horses:

Why IVA? – problems of myopathy in dependent limbs after volatile anesthetics (do intravenous agents maintain BP); do intravenous agents offer improved recovery; what agents are available and suitable for equidae and other large animals?

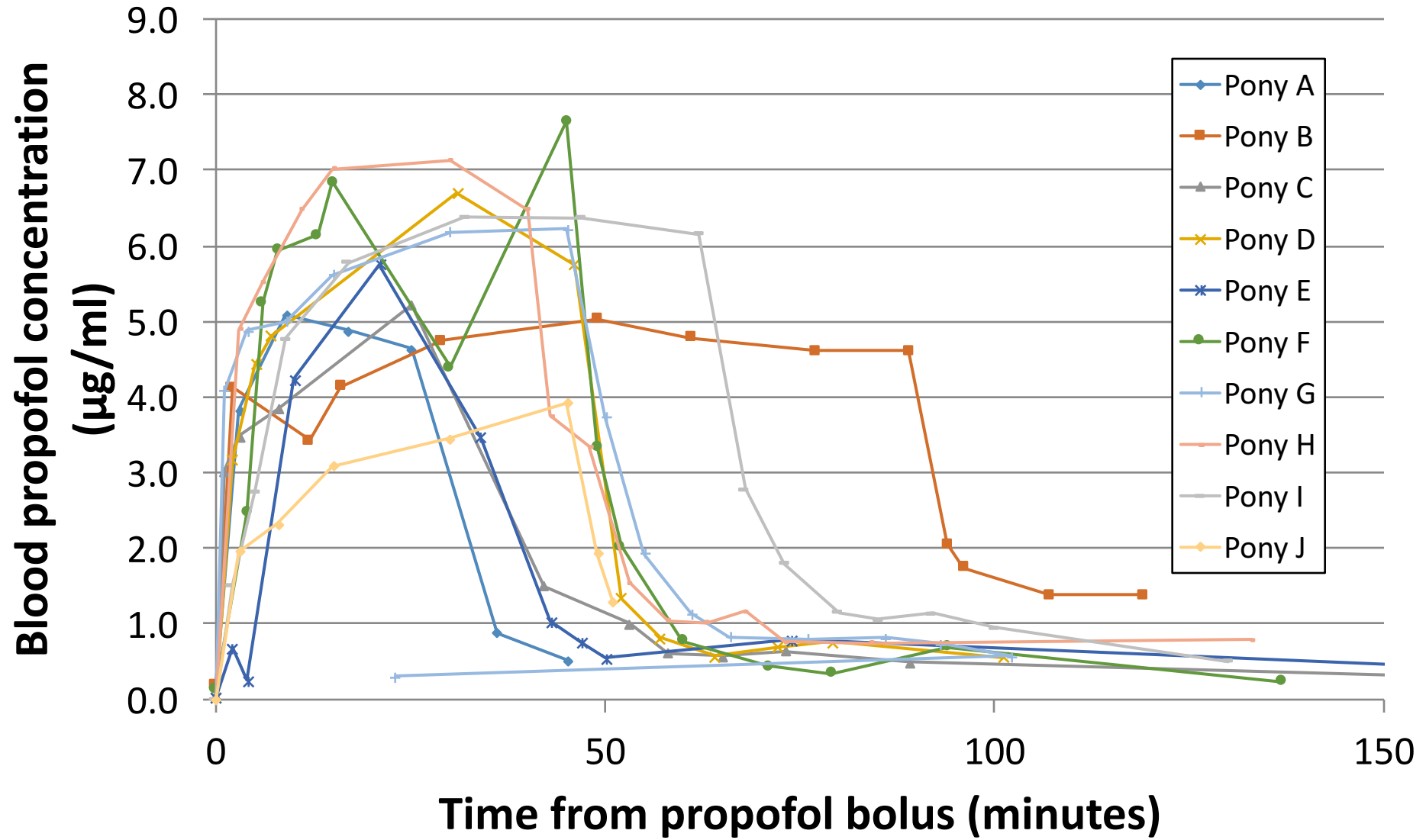
BUT WHAT DO WE KNOW ABOUT DRUG KINETICS and DYNAMICS?

Pelorus 1000 propofol measurement system



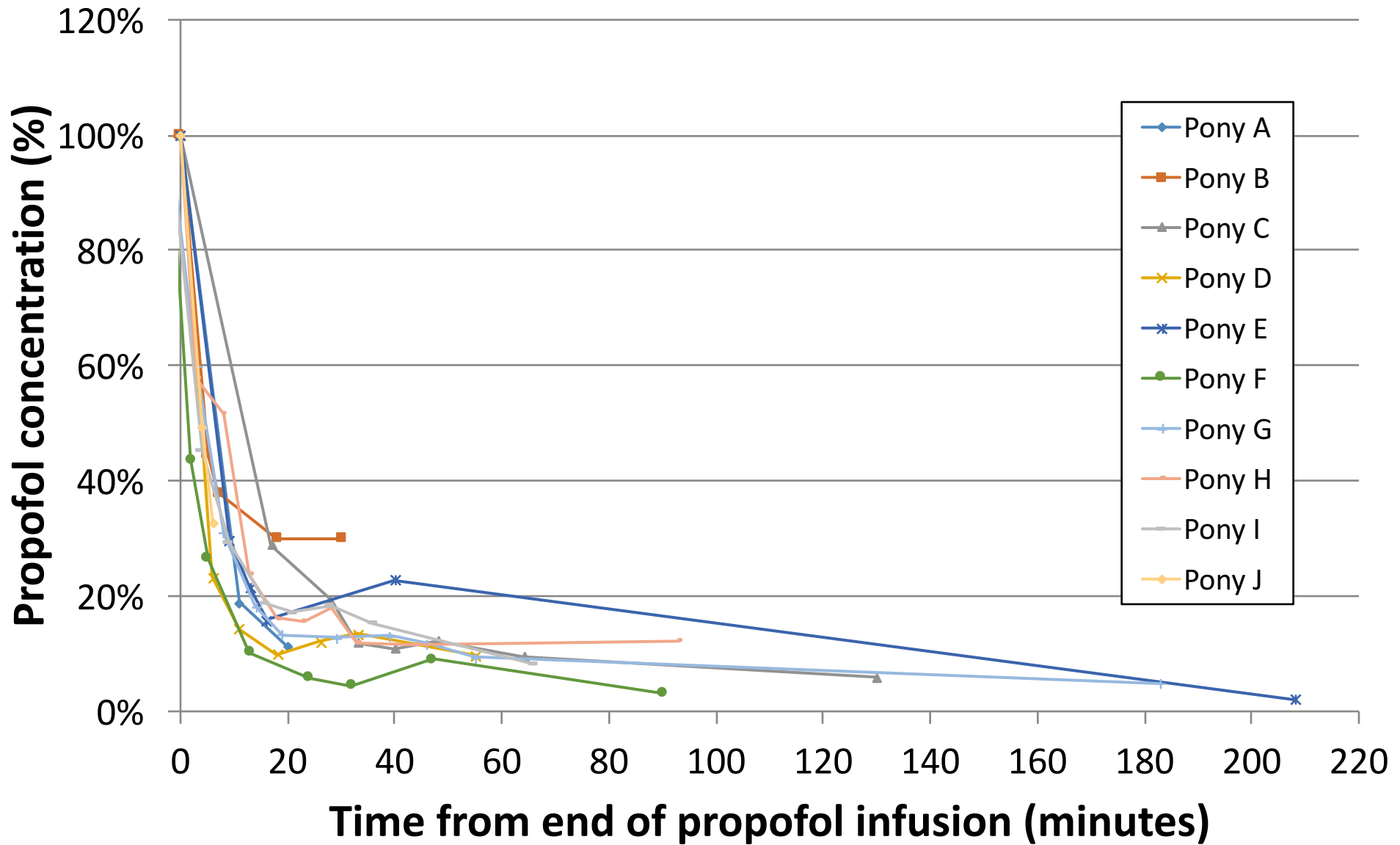
Study examining the pharmacokinetics of propofol by infusion in ponies.

- Ten Welsh-cross ponies (weighing 135-300 kg) undergoing minor procedures were studied (premedication acepromazine 0.03 mg/kg and detomidine 0.015 mg/kg). Anesthesia was induced with ketamine 2 mg/kg and diazepam 0.03 mg/kg, and maintained with an infusion of propofol at an initial rate of 0.16 mg/kg/min for the first thirty minutes, after an initial bolus of 0.3 mg/kg and decreasing by 0.01 mg/kg/min every 30 minutes; and ketamine by infusion (20-40 µg/kg/min).
- Blood samples: collected prior to, during and after the infusion, and on assuming standing position.

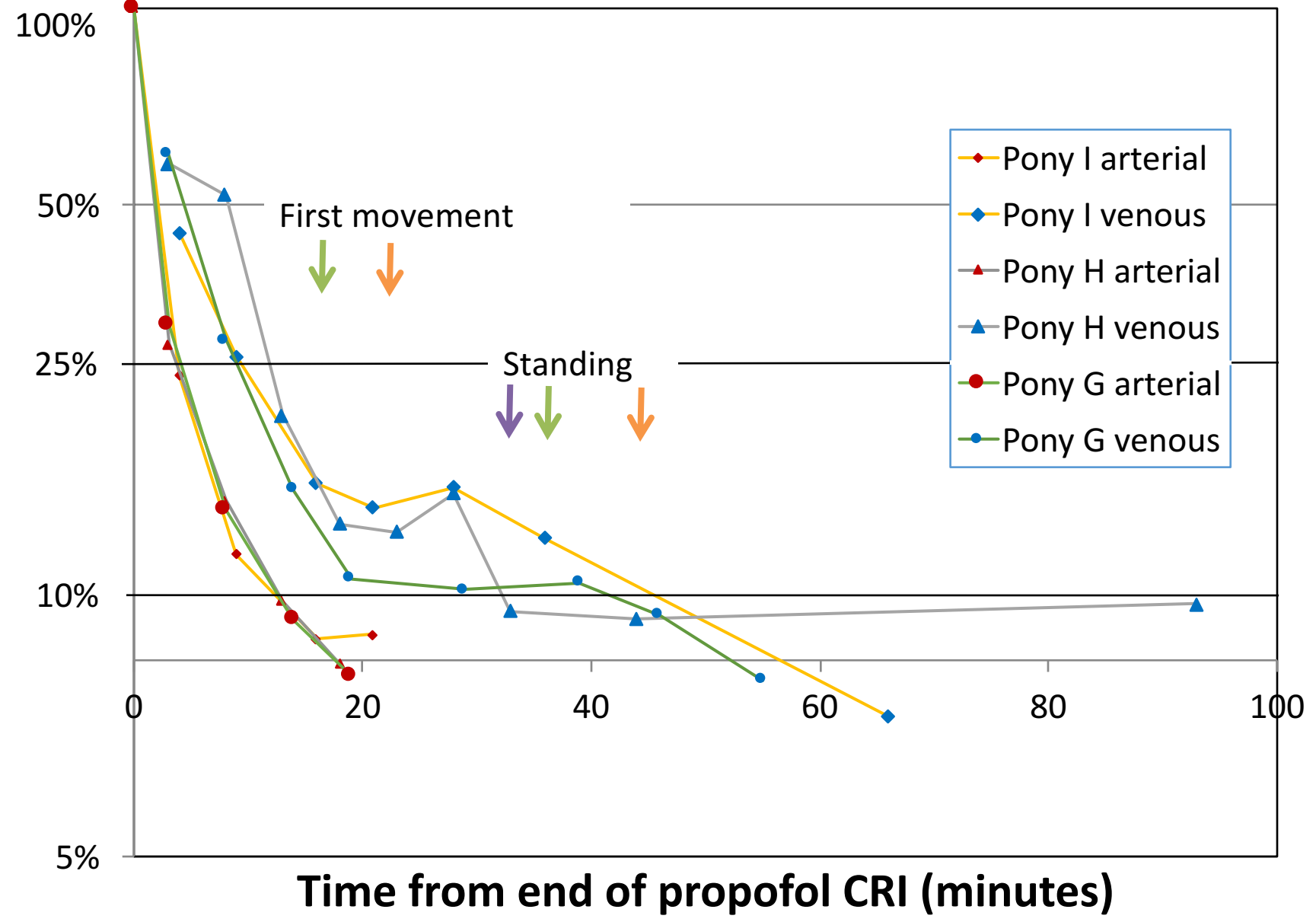


Results:

- Anaesthesia was uneventful; with the duration of infusion 31-89 min. Blood propofol concentrations during the infusion ranged between 1.52 and 7.65 $\mu\text{g}/\text{mL}$; pseudo-steady state concentrations 3.64-6.78 $\mu\text{g}/\text{mL}$.
- Blood concentrations on assuming standing position 0.75-1.40 $\mu\text{g}/\text{mL}$.
- Propofol whole blood clearance, volume of distribution and MRT_{inf} were 31.4 (SD 6.1) $\text{mL}/\text{min}/\text{kg}$; 220.7 (132.0) mL/kg and 31.9 (9.72) min respectively.



Propofol concentration normalised to final arterial reading



Blood propofol analyser: Pelorus 1000

- Rapid measurement of blood propofol concentrations (3-5 min.) – why might we need them?
- It may be useful for anaesthesia in young children and animals where kinetics are unknown; in disease states and where intercurrent therapies may affect propofol disposition. Are BIS and other EEG monitors reliable for all drug combinations? **The propofol analyser allows titration of propofol to a given concentration; and may be useful for anaesthesia in animals where kinetics are unknown; in disease states; and where intercurrent therapies affect propofol disposition**

Can we predict kinetics of drugs
in large animal species, to help
design delivery regimens?

Allometric scaling

Methods of scaling

Simple allometry (SA):

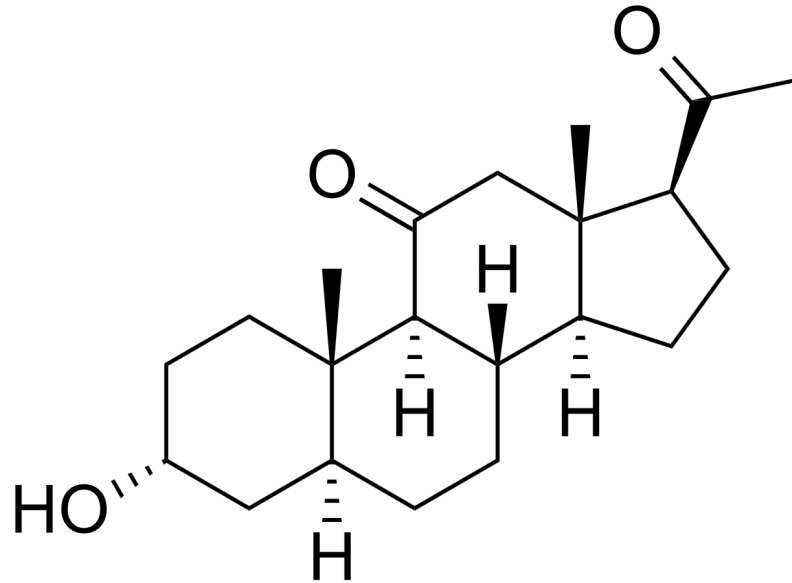
More advanced models:

- Product of maximum life-span potential (MLP) and clearance
- Product of brain weight and clearance
- Two-term power equation
- Incorporation of in-vitro data in in-vivo clearance
- Prediction of clearance in humans from in-vitro human liver microsomes or hepatocytes:
- Correction for protein binding
- Incorporation of molecular structure parameters
- f_u corrected intercept method
- Correction factors for renally secreted and biliary excreted drugs
- Monkey liver blood flow method

Basic allometric equation.

$Y = aBW^b$ where Y is kinetic parameter; 'a' constant and 'b' exponent.
BW = body weight.

Alphaxalone



Previously formulated in Cremophor EL, or propylene glycol-ethanol

New formulations:

2-hydroxypropyl- β -cyclodextrin

[Alfaxan-CD]: complexation ratio of Ax to HPBCD 1:4; damages kidney membranes; pancreatic cancer in rats.

Sulfobutyl ether- β -cyclodextrin

[Phaxan-CD]: CR 1:2 – hence less SBECD than HPBCD; ‘captisol’.

Study methodology:

- Kinetic data from 5 animal species (rat, rabbit, cat, dog, horse) and man in doses of 0.45 to 5 mg/kg.
- Modeling based on systemic clearance and apparent volume of distribution at steady state.
- Five formulations
- ALTHESIN - alfaxalone-alphadolone acetate in Cremophor EL
- Alfaxalone alone -n propylene-glucol ethanol; DMSO; cyclodextrin formulations
- Rat – Visser et al, 2000; 2002a and b; 2003
- Rabbit – Pastorino et al, 1979
- Cats – Whittem et al, 2008
- Dogs – Thomson et al, 1986; Ferre et al, 2006; Pasloske et al, 2009
- Foals – Goodwin et al, 2012;
- Horse – Goodwin et al, 2011
- Man – Simpson et al, 1978; Sear et al, 1979; 1981a and b; 1984
- [Rat – alfaxalone as PhaxanCD: Internet]

Sources of kinetic data:

- Rat – Visser et al, 2000; 2002a and b; 2003
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- Man – Simpson et al, 1978; Sear et al 1979; 1981a and b; 1984
- [Rat – alphaxalone as PhaxanCD: Internet]

Data analyzed using 'Rule of Exponents':

Based on simple allometry of clearance values:

Exponent 0.55-0.70 - SA

Exponent 0.71-0.99 - Cl.MLP better than SA or Cl.Br weight

Exponent >1.0 - Cl.Br weight best approach.

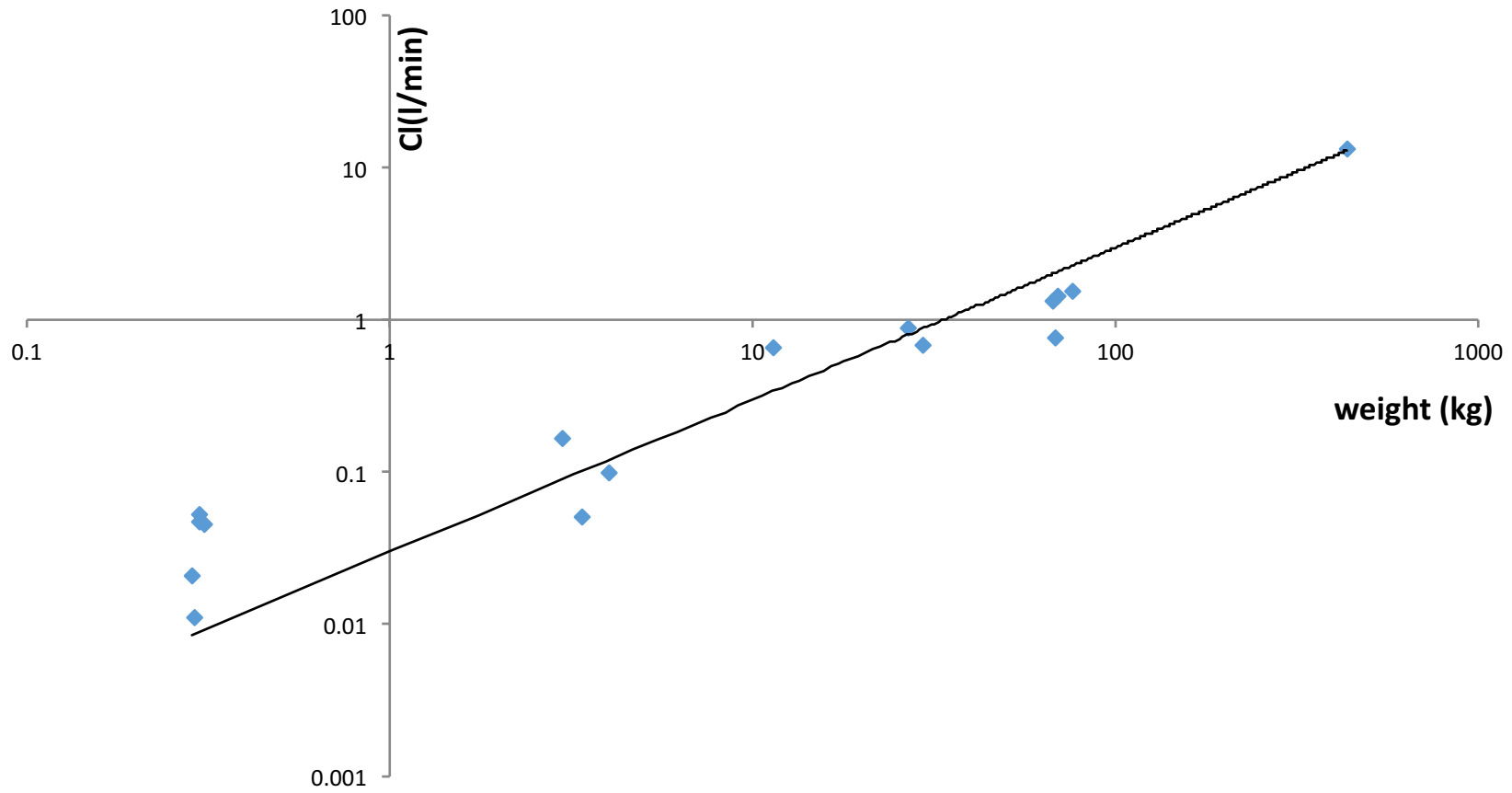
No use if exponent >1.3

- FEW STUDIES USING ALLOMETRY BASE ON unbound KINETIC VARIABLES!

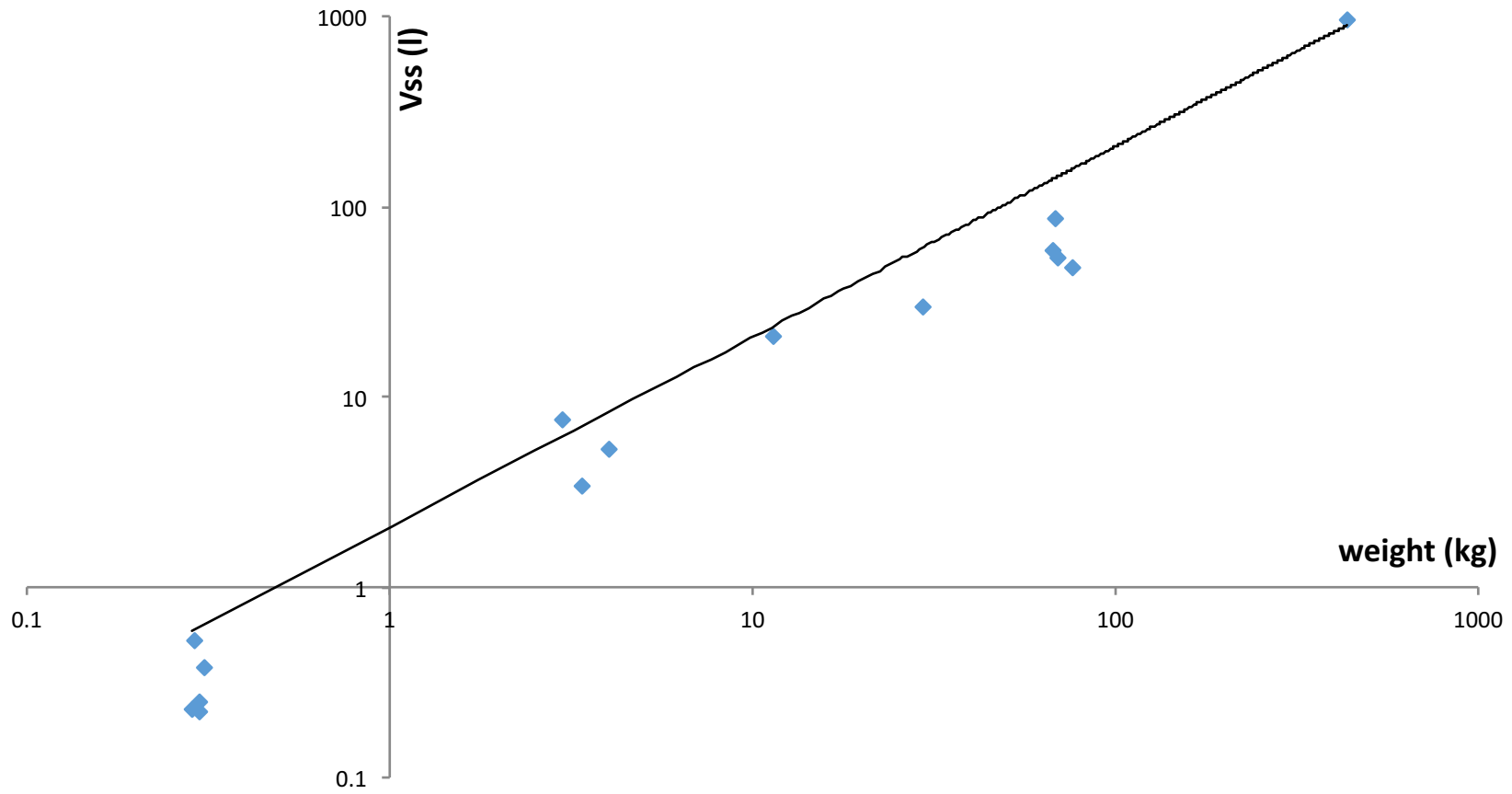
Results:

- Body weights of animals/ humans studied ranged between 285gm and 434Kg (log ratio 3.18).
- There were significant associations between body weight and Cl_{syst} and Vd_{ss} for alphaxalone.

Weight vs. Clsyst



Weight vs. Vdss



Allometric relationships:

When fitted to a simple power function:

$Cl_{\text{syst}} = 0.0625BW^{0.7461}$ ($r = 0.9548$); and $Vd_{\text{ss}} = 1.3025BW^{0.9740}$ ($r=0.9827$). There was no significant association between MRT and BW; nor any apparent influence of solvent on drug disposition.

Equations to overcome effects of neotomy by including terms for MLP or brain weight did not improve the fit of the clearance data.

Models for clearance based on MLP or Brain weight

Cl_{syst}.MLP = a.BW^b:

$$Cl_{syst} = 0.4801BW^{1.2685} (r=0.9772)$$

Cl_{syst}.brain weight = a.BW^b:

$$Cl_{syst} = 5.0099BW^{1.7081} (r=0.9827)$$

Neither model significantly better than SA alone

Validation of allometric model:

Validation methods:

Effect of equidae data:

	<u>Present model</u>	<u>Minus equidae</u>
Influence of horse on modelling (largest species)?	A 0.0626	0.0641
	B 0.7457	0.6952
Leave-one-out correlations	R 0.9549	0.9478
	R ² 0.8947	0.8675*

- * no significant difference between models

Conclusions:

- This study showed that relationships between body weight and the pharmacokinetic parameters of Cl_{syst} and Vd_{ss} in 5 animal species and man for alphaxalone can be described by a simple allometric equation. The values for the exponent b are in good agreement with the expected values of 0.75 for clearance and 1.0 for Vd_{ss} .
- Use of more complex allometric equations relating body weight and pharmacokinetic parameters are not needed for high hepatic extraction ratio drugs such as alphaxalone, as they do not improve the predictive fit of the model.
- The study also predicts that cyclodextrin formulations of alphaxalone, when studied in man, will show kinetics that are not significantly different from those seen with the Cremophor formulation.

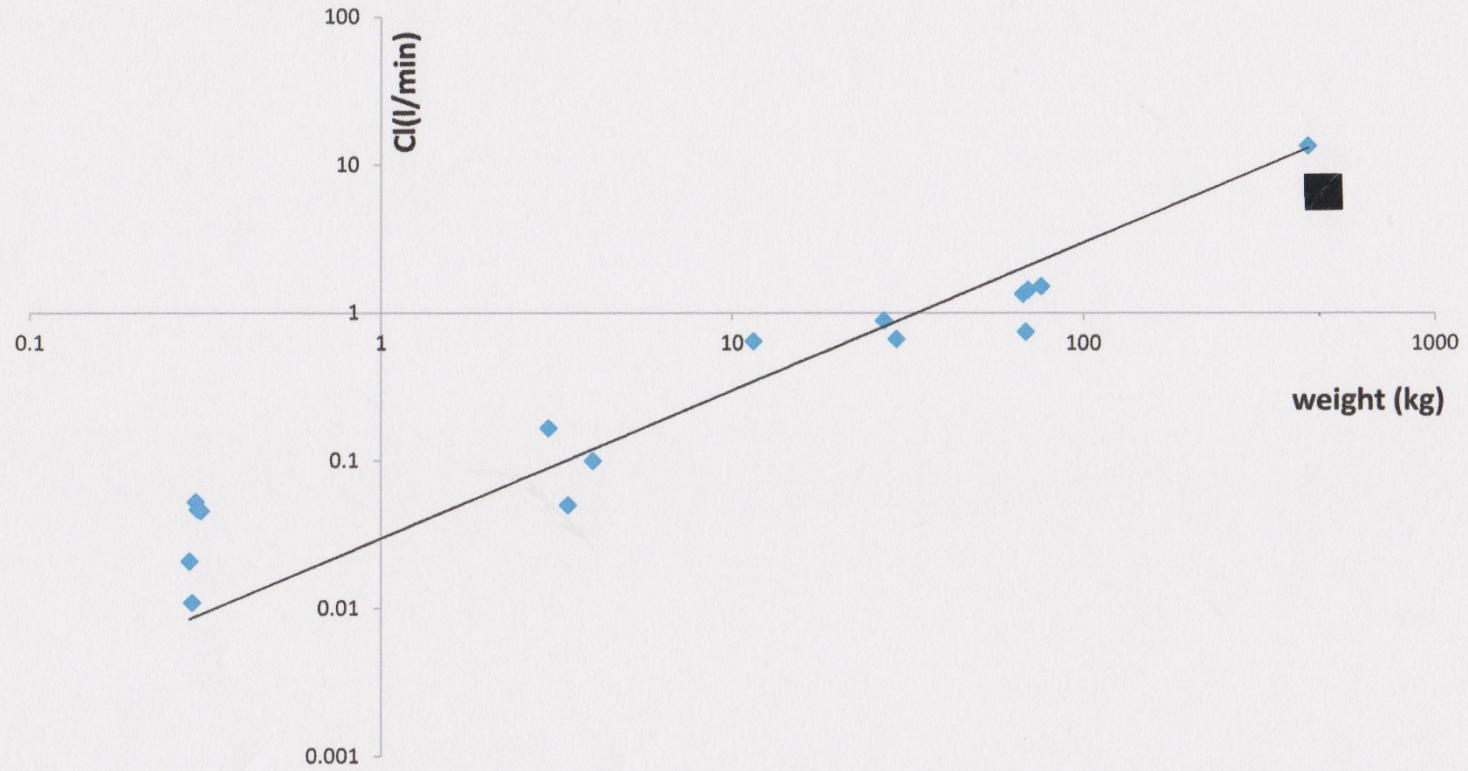
Caveats to successful use of allometry

- Adequate range of weights and kinetic values (> 3 orders)
- Need to predict between rather than within species
- Results are subject to both measurement and biological variability

Kinetics in camels - alfaxalone



Weight vs. Clsyst



How would you like to anesthetize these with TIVA?

Amur Leopard



Sumatran Tiger



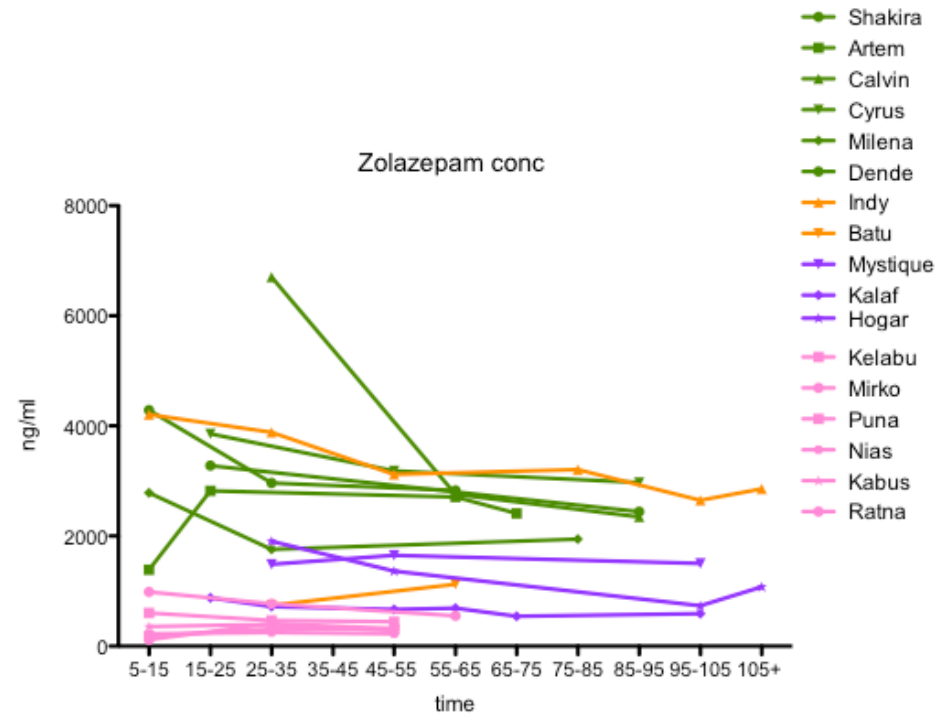
Comparison of tiletamine and zolazepam pharmacokinetics in tigers and leopards: do species differences account for adverse effects in tigers?

Lewis JC, Teale P, Webber G, Sear JW, Taylor PM

Vet Journal 2014; 201: 302-306

Protocol for routine examination/ minor surgery:

- Tiletamine/ Zolezapam (Telazol) im. to 8 tigers and 9 leopards
- Medetomidine premedication 20-30 ug/kg to 3L and 2T
- Blood sampling and drug measurement by HPLC:MS
- Peak concentrations: 9-33 minutes



LEOPARD Z
TIGER Z
LEOPARD ZM
TIGER ZM

Results and conclusions:

- Abnormal recovery in one tiger (hind-limb weakness at day 5; response to im midazolam): were there kinetic reasons (uremia, old age)?
- ZT ratio generally <5 ; with no difference between leopards and tigers
- Z metabolism: primarily by demethylation; but also evidence of hydroxylation in L
- We conclude that the occurrence of abnormal neurological signs at 2-4 days after recovery in some tigers does not appear due to major kinetic differences between T and L.

So, what possible advances for the next 37 years?

- New drugs? – but will need to show fast $t_{1/2}$ k_{e0} and appropriate slope relationships (γ) for PK-PD plot; minimal CVS (and RS) effects; cerebral (and cardiac) protection; ideally metabolism by hydrolysis, with no active metabolites, and no toxic effects of drug solvents.
- Development of online measurement of drug concentrations – perhaps the (pharmacologically active) free drug concentration.
- Closed link control of anesthesia; but to which ‘depth of anesthesia’ monitor?