Validation of *in vivo* Mouse PK Assay by Mixed Effects Modelling: Estimation of Between-Study Variability.

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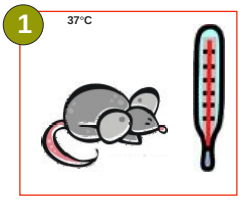
AIM:A capillary bleed sampling technique was evaluated in-house. This technique allowed the sampling of multiple time points from a single mouse. Reproducibility, at least for this compound, would be assessed if the majority of variability could be assigned to inter-animal variability rather than inter-study variability.

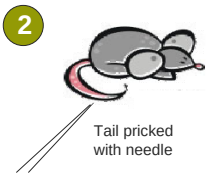
METHOD: The data were analysed in NONMEM VI, the NLME toolbox in R and WinBUGS for a comparison of results.

RESULTS: Different Software gave different results. NONMEM estimated mainly inter-individual variability whilst WinBUGS attributed variability more evenly to the two levels of random effects

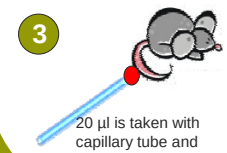
DATA COLLECTION



Mouse placed in warming chamber for 5 mins before sampling



Tail pricked with needle

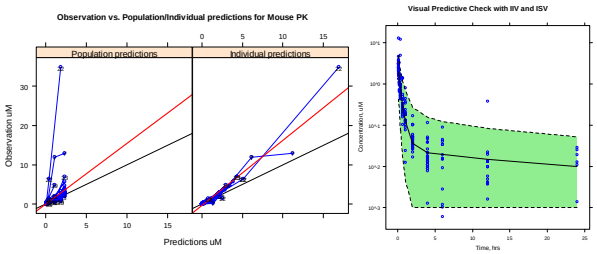


20 µl is taken with capillary tube and diluted with 60 µl of water

1. The reproducibility of this technique was investigated by carrying out **7 in vivo studies**. Between **4 and 24 animals** were included in each study
2. The variability in the data may then be partitioned into inter-study and inter-animal.
3. Five time points per animal in two staggered groups:

Animal 1	Animal 2	Animal 3	Animal 4
5 min	5 min		
		10 min	10 min
20 min	20 min		
		40 min	40 min
1 hr	1 hr		
		2 hr	2 hr
4 hr	4 hr		
		6 hr	6 hr
12 hr	12 hr		
		24 hr	24 hr

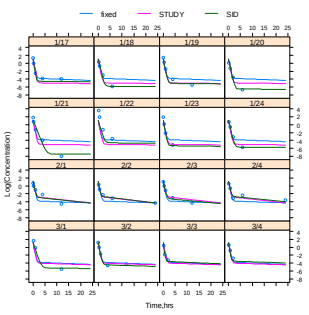
NONMEM



Parameter	Typical	IIV	ISV
CL (L/hr/kg)	5.47	54%	0
V1 (L/kg)	3.52	39%	0
V2 (L/kg)	95.3	80%	50%
Q (L/hr/kg)	5.74	56%	0
Err Prop	39%		
Obj Fun	FOCE	-498.9	-525.5

1. **Study** is placed at the individual level
2. **Animals** are "occasions" within a particular study
3. **Model**: Two compartment model
4. **Between Animal** variability estimated as larger than **Between Study**

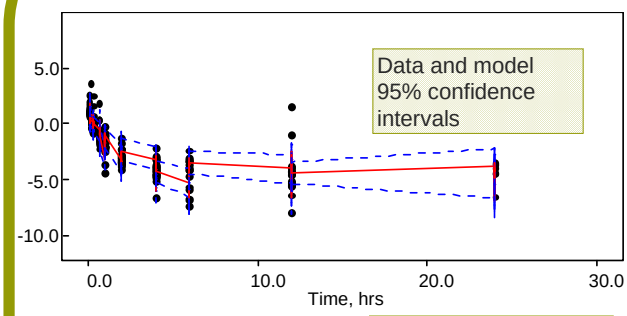
NLME (in R) R



Parameter	Typical	IIV	ISV
CL (L/hr/kg)	4.95	40%	0
V1 (L/kg)	3.32	0	0
V2 (L/kg)	109	0	80%
Q (L/hr/kg)	5.21	72%	0
Err Prop	58%		

1. NLME toolbox uses Lindstrom-Bates Method
   - Linearisation around current estimate of random effects
2. FOCE in NONMEM method is similar
3. **Multiple levels of Random Effects**
4. Results similar to NONMEM
5. **Convergence** was very sensitive to initial parameter values

WinBUGS



Parameter	Typical	IIV	ISV
CL (L/hr/kg)	2.41	67%	53%
V1 (L/kg)	3.88	55%	41%
V2 (L/kg)	199	69%	54%
Q (L/hr/kg)	6.22	46%	44%
Err Prop	75%		

1. **PKBugs** 2-compartment model.
2. **Five chains** were run for **10000 iterations**
3. All Chains converged
4. **Median** Parameter values reported

CONCLUSIONS: Overall the results suggest that the variability in the data is largely inter-individual. In conclusion the assay is sound.

1. **The analysis is readily applicable, though there are a number of pitfalls – especially with respect to the NONMEM implementation of the statistical models using separate random effects for each animal.**
2. **The results also show that with this quantity of data (48 animals) and an unbalanced design, different parameter estimates may be obtained by using different methods.**
3. **The results however point towards a more sophisticated use of data when planning drug discovery life-phase activities. Taking a Mixed Effects approach allows between animal and study differences in response to be better understood.**

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