National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Overcoming the barriers for uptake of microsampling techniques in regulatory toxicology

European Bioanalysis Forum, November 21st 2013.

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Head of Innovation and Translation

Pioneering Better Science

Who are we?

- Independent, scientific organisation
- Established in 2004 by Government
- Role is to support the UK science base through the application of the 3Rs
- Budget ~£6.5 million per annum
- Team of 20 staff
- www.nc3rs.org.uk

NC 3Rs

abpi BBSRC bioscience for the future defra Department for Environment Food and Rural Affairs

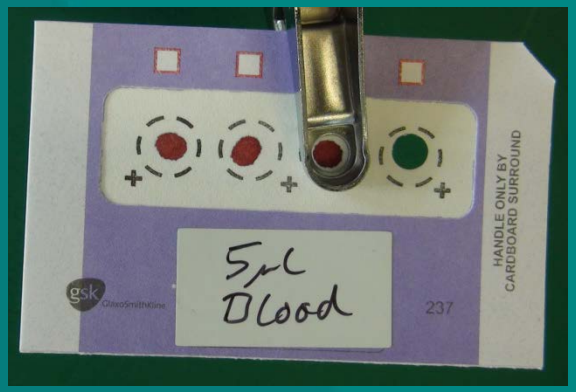
Dow gsk GlaxoSmithKline Home Office

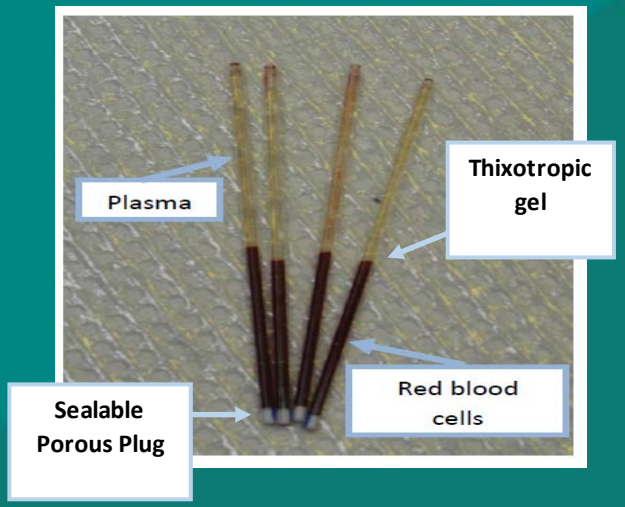
syngenta

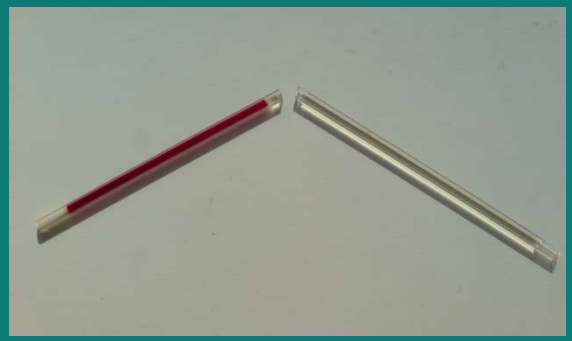
MRC Medical Research Council SC Johnson A FAMILY COMPANY Unilever

wellcome trust Technology Strategy Board Driving Innovation

Use of microsampling techniques in drug development







Overcoming barriers to use of microsampling

Scientific question

- Can we use microsampling to relate exposure to toxicity by sampling main study animals?

How is this being done currently?

- Exposure not being linked to toxicity and large numbers of satellite animals are being added to measure toxicokinetics (rodents)

Use of microsampling techniques in drug development

- "The largest influence on numbers, particularly rodents, is collection of samples for toxicokinetic analysis" (*Sparrow et al Regulatory Toxicology and Pharmacology 61 (2011) 222-229)*

Table 1: Example of a study design for a six month rat study with assessment of recovery and satellite animals

Dose group	Low	Medium	High	Control
No. of animals	15M + 15F	15M + 15F	15M + 15F	15M + 15F
No. of TK satellites	9M + 9F	9M + 9F	9M + 9F	3M + 3F
No. of recovery**			5M + 5F	5M + 5F
Maximum total for one study				200*

Table 3: Example of minimised study design for a six month rat study with assessment of recovery without separate satellite animals

Dose group	Low	Medium	High	Control
No. of animals	15M + 15F	15M + 15F	15M + 15F	15M + 15F
No. of recovery			5M + 5F	5M + 5F
Typical total for one study				140*

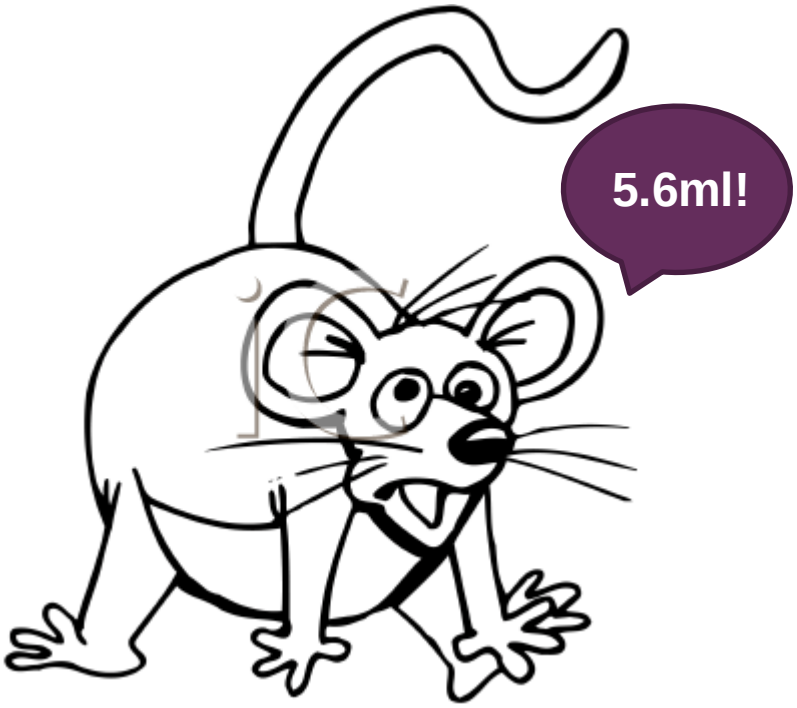
* This design includes toxicokinetic sampling from main study animals rather than the use of toxicokinetic satellite animals

NC 3Rs

You Want How Much?

Toxicologist

DMPK



Slide courtesy of Sue Sparrow, GSK

Delivering the Science but commitment to 3Rs

Slide courtesy of Sue Sparrow, GSK

Use of microsampling techniques in drug development

- Potential to refine procedures e.g. reduced blood loss, alternative sampling sites, quicker & less stressful sampling (e.g. warming)
- Better scientifically as toxic effects can be related to exposure in the same animals
- Business benefits i) reduced compound needed, ii) reduced resource, iii) fewer stages between sampling collection and analysis (reduced error)
- What's stopping us?

Microsampling survey

- Workshop in May 2013 with 80 industry representatives
- Survey conducted to identify barriers to use of microsampling
- 77% of companies currently using microsampling and 100% want to

Figure 1a

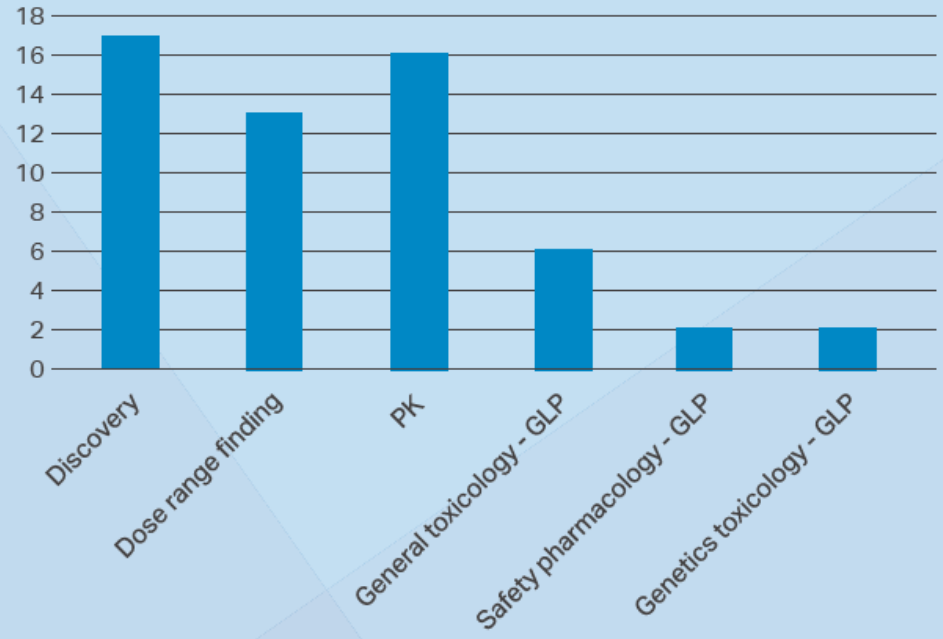
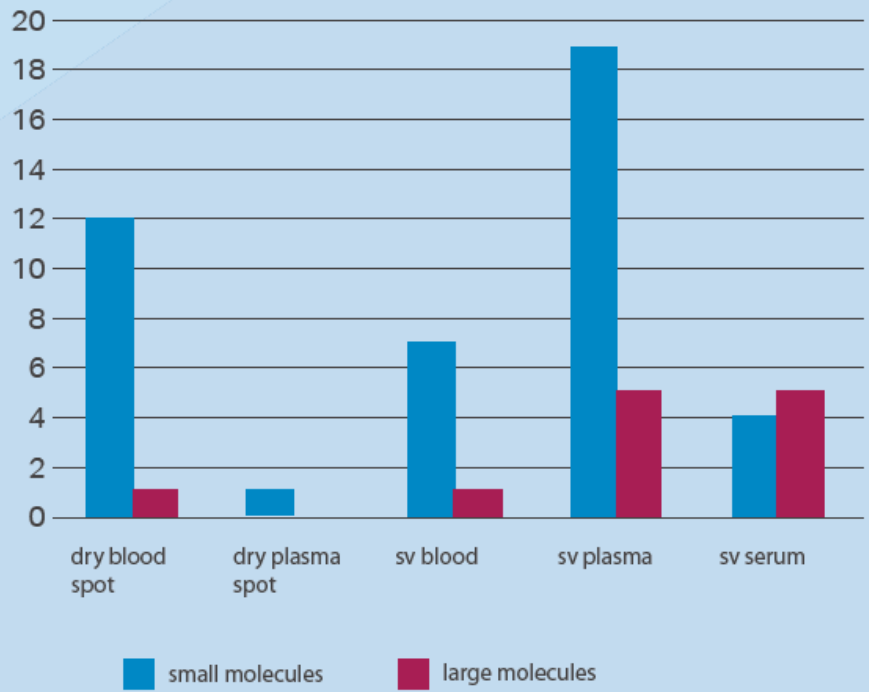
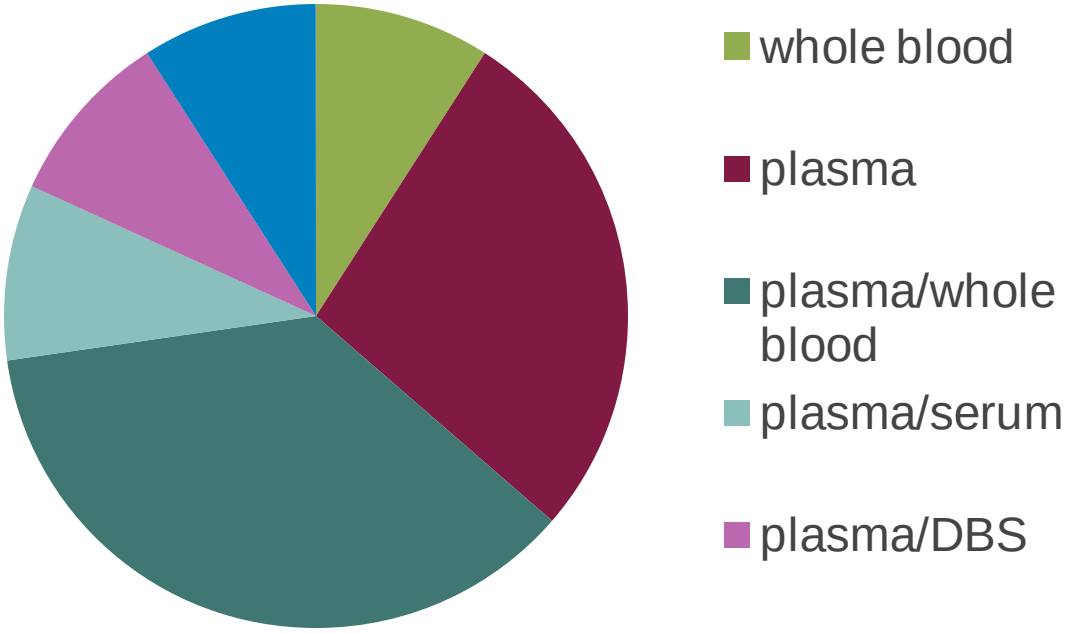


Figure 1b



Microsampling survey

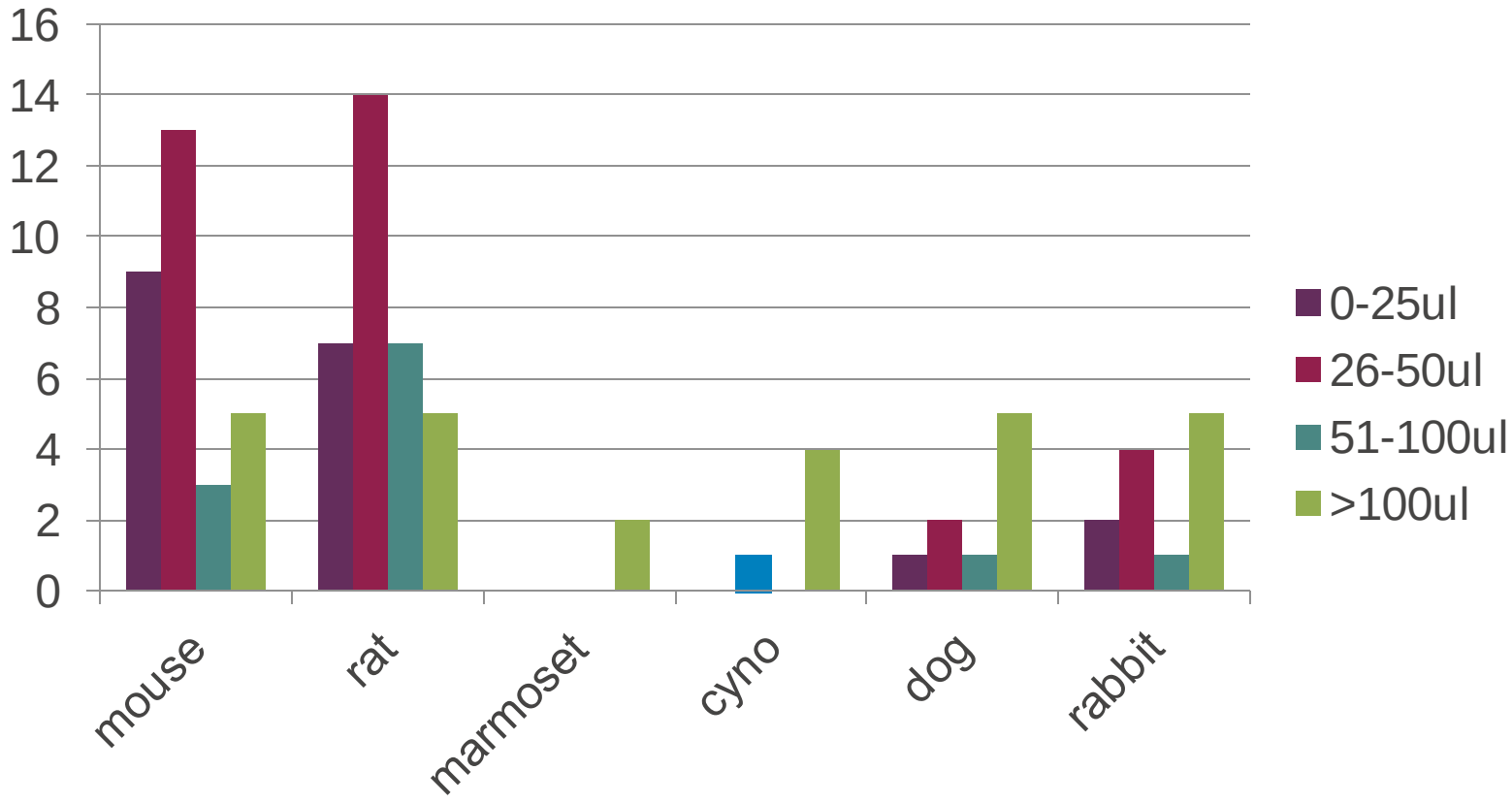
- What samples are typically processed for quantitative bioanalysis? E.g. blood, serum, plasma, dried (e.g. blood spot)



Sample type	No.
plasma	10
whole blood	6
serum	1
DBS	2

- Only one company not using plasma

Sample Volume: What is micro?



Consensus definition: a microsample may be defined as a small volume of blood which would allow serial or composite sampling of main study animals with limited impact on other study parameters.

Typically a normal sample would be ~200ul (range 100-800ul) and a microsample 25-50ul (range 10-100ul)

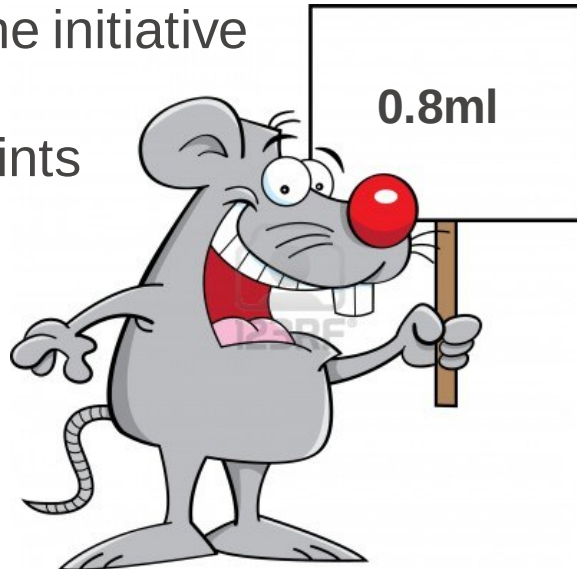
Microsampling survey: methods and practicalities

- Needle size (21G-27G)
- Warming (social huddling to up to 30 minutes in hotbox)
- Sampling of controls (two samples to treat exactly the same)
- Restraint (sit on arm to tubes)
- Sample and dilution

Dilution factors	Diluents	Primary sample
No dilution		
10+10ul	Sodium citrate	50:50 primary: aliquot
10+30ul	PBS (small mols)	
20+80ul	Protein precipitater plasma	
10+90ul	Plasma:plasma	
10+900ul	Blood:blood	
	Assay buffer containing BSA (large mols)	

Overcoming barriers to use of microsampling: NC3Rs/cross-company strategy

- Most significant remaining barrier to uptake is sampling from main study animals in GLP toxicology studies
- Evidence base needed to show that sampling from main study animals will not compromise other study end-points
- Strategy to share data and develop cross-company publication/s from combined data on microsampling agreed
- 42 workshop participants signed up to progress the initiative
- Address regional differences in regulatory viewpoints e.g. FDA, MHRA, Japan



Investigative Microsampling Study

Adult Rat

- Crl:WI(Han)
- Approximately 10 weeks of age

Sampling method	Average Start weight (g)	Average end weight (g)
Group 1 no-sample control	M – 341 F – 225	M – 360 F - 233
Group 2 large volume sampled	M – 332 F - 218	M – 343 F - 229
Group 3 microsampled	M – 344 F - 220	M – 358 F - 225

- 3 groups – 10/sex/group
- All animals vehicle dosed – 5 mL/kg - Water containing 0.5% w/v hydroxypropyl methylcellulose and 0.1% w/v polysorbate 80
- Group 2 and 3 sampled 6 time points (32ul vs 200ul) day 1 and day 14

AstraZeneca

Nicola Powles-Glover, November 2013 [Nicola.PowlesGlover@astrazeneca.com]

Results – Clinical Pathology

	Group 1 un-sampled	Group 2 Large volume	Group 3 Microsampled	Historical range of haematological parameters For adult rats
Haemoglobin				
M	15.5	13.6***	15.3	13.61 – 17.06 g/dL
F	15.0	12.7***	14.4*	12.60 – 16.07 g/dL
Haematocrit				
M	0.47	0.42***	0.46	0.40– 0.52 L/L
F	0.46	0.40***	0.45	0.37 – 0.49 L/L
Red Blood Cells				
M	8.59	7.49***	8.49	7.27 – 9.54 10^12/L
F	8.10	6.76***	7.87	6.58 – 8.64 10^12/L
Reticulocytes %				
M	2.6	3.4***	2.6	1.7 – 4.5 %
F	2.9	3.7**	3.2	1.9 – 5.4 %
Neutrophils				
M	0.8	1.32**	1.2	0.50 – 2.57 10^9/L
F	0.84	0.84	0.67	0.31 – 1.89 10^9/L
Monocytes				
M	0.10	0.17**	0.15*	0.05 – 0.30 10^9/L
F	0.11	0.12	0.11	0.04 – 0.24 10^9/L
AST				
M	69	96**	70	54 – 134 IU/L
F	67	84**	69	46 – 128 IU/L
GLDH				
M	6	11*	8	2 – 20 IU/L
F	5	8*	6	2 – 20 IU/L

Interpretation & Recommendations

- Use main test animals not satellites for collection of full TK profile
- BUT might be minor changes in haematology
  SO
- Sample control animals as well as compound dosed animals

Technical recommendations and refinements

- Limit hot box exposure to only 1 - 2 minutes

- Un-restrained on vet bed or handlers arm, OR hand held (worse case scenario) for microsampling.

Overcoming barriers to use of microsampling: where are we up to?

- Paper accepted in Drug Discovery Today
- Questionnaire sent to 42 participants on practical aspects of microsampling
- Technical tips/do's and do not's
- Collecting individual company evidence to provide a review on what effect microsampling has on animals, particularly related to key study endpoints (e.g. AZ data)
- Link into regulators and ICH

The Impact – 3Rs

Study Type	Conventional design with satellite animals	Micro-sampling design	Animal Reduction
Dose Ra Rat			
One Mor Toxicity			
Three M Toxicity			

Financial impact

One company estimated about $350,000 saving/year

- Included: reduction in animals, reduction in personnel (sampling), less compound
- Excluded: calculations for reduced space, energy, time and personnel e.g. 12 x fewer animals – weighing, moving cages

Totals: 184 *vs* 310

Thank you

- Everyone at the workshop and who filled in the questionnaire

- Susan Sparrow, GlaxoSmithKline
- Neil Spooner, GlaxoSmithKline
- Sally Robinson, Astra Zeneca
- Amanda Wilson, Astra Zeneca
- Tim Sangster, Charles River
- Simon Chivers, Novartis
- Dan Gliddon, Huntingdon Life Sciences
- David Mitchell, Huntingdon Life Sciences

- Nicola Powles-Glover, Astra Zeneca
- Jane Stewart, Astra Zeneca

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