

Report by Samuel Anees-Hill

## **Symposium 6: Microsampling in Toxicology: Maximising the scientific, business and 3Rs advantages.**

**Chairs: Dr Fiona Swell (NC3Rs) and Dr David Jones (Independent. Ex-MHRA).**

### **≤ 100-word summary**

Microsampling provides a relatively new and exciting avenue related to blood sampling methodology. The principle involves the extraction of a much smaller volume of blood (100 microlitres or less) compared to traditional techniques, whilst still retaining full, and often enhanced, analytical capability. This provides refinement-related benefits in animal testing, through the reduced need for large volumes, and the capability of using less-invasive and painful sampling sites. Industry uptake is high, although there is variability in its scope. The method also has utility in patient settings. This refinement can lead to happier animals, which in turn results in good science.

### **Preclinical to clinical use of microsamples – start small, stay small. - Dr Neil Spooner (Spooner Bioanalytical Solutions)**

Recent advances have made possible the use of microsampling, a blood-sampling technique which enables the analytical use of blood samples of 100 microlitres or less. Microsampling can provide both financial and ethical benefits in their use. The technique is less invasive and can provide higher quality data. With use of smaller volumes, repeated sampling is more feasible and tends to display better correlations at the analytical stage. Samples may also be taken for other purposes with minimal total waste of the sample.

One of the principle benefits of this technique is a reduced cost, although the total cost of consumables remains higher. In addition to the ethical benefits of taking less blood, the smaller volume also allows for collection from less invasive sites, which can reduce the potential stress experienced by the subject. Important considerations remain, however, the use of microsampling in paediatric and home settings can significantly benefit from this technique.

### **Current use, 3Rs benefits and barriers for microsampling: results from survey of academia, pharmaceutical, agrochemical and CRO sectors - Dr Helen Prior (NC3Rs)**

In 2019, NC3Rs distributed a survey to an array of labs/companies that utilise/can benefit from the use of microsampling. This involved a series of questions probing whether the respondent uses these methods, how they use them, and their reasons for using them or not. This was written up into a 2021 cross-section study involving 54 surveys in total. Most responses were from pharmaceutical companies, but also included academia and CRO from the UK, EU and USA.

Results of the survey showed the majority of companies do use microsampling (for any purpose), with some using this for non-GLP studies only. There was a myriad of reasons for use of microsampling, with small molecules being a prominent use case. Reasons for not utilising microsampling was attributed to regulation issues, the need for larger samples, validation requirements, and a lack-of-interest from sponsors. A key message from the study is that there is good awareness of microsampling as a technique, although there is high variability in how the technique is applied.

### **Is microsampling applicable for large molecules and other drug modalities? - Dr Amanda Wilson (AstraZeneca, UK)**

AstraZeneca utilise capillary plasma microsampling as an approach to sampling. Recent validation efforts displayed good analytical results with good consistency involving low coefficients of variation (CV), with a criteria of <20% variation and only a couple of validation samples breaking above 15%. Recent feasibility studies utilising the Neoteryx Mitra specimen collection kit showed good calibration curves and concordance with existing methods with use of 20 microlitre samples. With a single analysis, the matrix requirements are halved, and sensitivity is good. The benefits to use of microsampling techniques are ethical, financial, and scientific and worth pursuing.

### **Incorporating microsampling for agrochemical TK data to improve risk assessment - Derek Angus (Syngenta, UK)**

By using microsampling, more blood samples from the animals can be acquired, which can be useful for the two types of relevant assessment include risk and hazard. Dose proportionality assessments present one area for use of this sampling technique, in areas such as acute, sub-chronic, neurotoxicity and reproductive toxicology. Such assessments require the use of many samples (up to 14 samples, per animal, per week), which is made possible by the use of microsampling, which is also shown to produce enhanced data quality, in part because of the number of samples that can be obtained using this method. Overall, there's a growing body of evidence to show that microsampling has no adverse effect on animals which abides by the refinement principle of the NC3Rs.

Microsampling of main test animals with no detriment to clinical pathology parameters - **Carol Strepka** (CRL, UK)

This study involved the mining of historical toxicology study data with an aim of analysing differences in traditional sampling methods versus microsampling. Comparing macrosampling to microsampling shows a large difference in the amount of blood needed, with up to 9ml total required from each animal, including an additional 2.4ml for a clinical pathology sample. Microsampling requires much less, with 96 microlitres taken per animal (10x less). Analysis of the historical data also involved comparisons of microsampling vs macrosampling, and each of these versus no TK (toxicokinetics). Results showed no differences in clinical chemistry parameters between animals. In haematology, two-thirds of samples showed no differences for haematology parameters. The third that showed differences were of little biological relevance. In conclusion, macro- and microsampling in adult rats does not compromise the clinical pathology end points, which provides benefits to animals, and also a reduction in the need for husbandry consumables, test items and reagents.

Animal welfare benefits of microsampling for any purpose - **Dr Hollie Blunt** (Sequani, UK)

Replacement remains perhaps the most significant goal, although this isn't quite possible yet. In the meantime, reduction and refinement are seeing good progress. In terms of refinement, microsampling can utilise less invasive sites that cause stress and pain for small animals in particular. Benefits of microsampling include reduced blood loss, stress and pain, and the use of routes without need for anaesthesia (which can result in breathing difficulties for smaller animals), or products that direct blood to the site (i.e. in rabbit ears). These methods can benefit small creatures such as rodents the most, although this can benefit larger animals too.

The use of a closed sampling system contributes towards procedural refinements, with samples able to be taken from small superficial veins, which can particularly help with animals susceptible to stress, such as mini pigs. In all, such refinement can lead to happy animals, which results in good science.