Determination of Microsome and Hepatocyte Fuinc Values for Multiple Species Using the TRANSIL Microsomal Binding Kit

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Purpose
The unbound fraction (Fuinc) of compound in in vitro metabolic stability incubation is an important parameter for accurate determination of intrinsic clearance (Clint) as opposed to the apparent intrinsic clearance (Clintapp). Fuinc values can be incorporated into in vitro in vivo extrapolation (IVIVE) from microsomes and hepatocyte incubations using the well stirred model to improve the prediction accuracy of clearance.

Methods
Traditionally Fuinc measurements for hepatocytes are determined by equilibrium dialysis which requires the addition of CYP and UGT inhibitors (AstraZeneca’s method) or “dead” hepatocytes to remove the metabolic component from the experiment which can compromise the analysis.

Results
We found that the amount of phospholipid present in microsomes (1 mg/ml) and hepatocytes (million cells/ml) does not follow a 1:1 relationship. A good correlation was observed between human microsome Fuinc values using TRANSIL and dialysis methods. TRANSIL rat hepatocyte Fuinc values correlated poorly with results for dialysis incubation, while the TRANSIL rat hepatocyte Fuinc values correlated highly with predictions based on the Kilford method. The poor correlation between TRANSIL and dialysis rat hepatocyte Fuinc measurements may be due to the failure to fully prevent metabolism occurring in rat hepatocyte dialysis experiments.

Conclusion
The common practice of using rat or human hepatocyte Fuinc values in the in vitro in vivo scaling of dog clearance should be used with caution. The TRANSIL binding kit can be used for determining Fuinc values in both microsomes and hepatocytes from a single set of data. It provides a quick method for generating data to update the Fuinc predictive models for compounds that do not predict well i.e. lipophilic acids and spot checking predictions as compounds progress towards development. The TRANSIL Microsomal Binding kit measures the affinity of drugs to human microsomal membranes using beads with a single phospholipid bilayer reconstituted from synthetic lipids resembling the natural composition of human liver microsomes. The amount of phospholipid present (mg phospholipid per mg microsomal protein) in human microsomes is used in the Fuinc calculation. This raises the possibility of determining Fuinc values for microsomes and hepatocytes in multiple species from a single experiment after correction for the amount of phospholipid present.