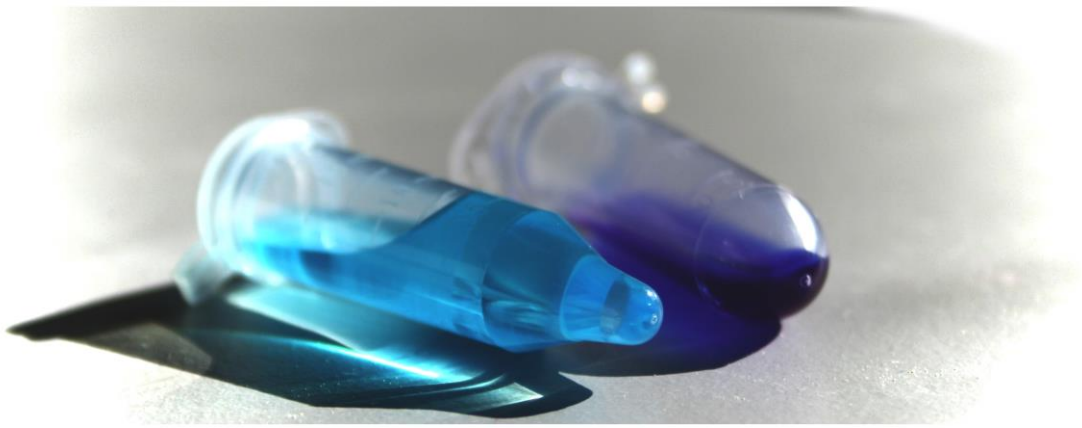

# In vitro drug metabolism: for the selection of your lead compounds

## S9 Stability Assay:

The so called S9 fraction of liver homogenate contains the vast majority of both membrane-bound and soluble phase 1 (i.e. oxidative, reductive and hydrolytic) and phase 2 (i.e. conjugative) enzymes involved in drug metabolism. Thus, the S9 stability assay, compared to our microsomal stability assay, more broadly assesses the overall hepatic metabolism of the test compound.

Liver S9 enzymes include, among the others, cytochrome P450s (CYPs), glucuronosyl transferases, sulfotransferases, esterases, alcohol and aldehyde dehydrogenases and aldehyde oxidases, the latter being currently recognized to be heavily involved in the metabolism of chemicals containing azaheterocyclic rings (Parkinson et al., 2013; Lepri et al., 2017).

Our standard S9 stability assay examines the disappearance over time of a test compound in incubations mixtures containing the liver S9 fraction, both in the presence and absence of the cofactors needed for CYP-mediated drug oxidations (i.e., *nicotinamide adenine dinucleotide phosphate*; NADPH) and glucuronidations (i.e., uridine diphosphoglucuronic acid; UDPGA); assay mixtures need to be supplemented with other appropriate cofactors to investigate other metabolic pathways (e.g. sulfonation and alcohol and/or aldehyde dehydrogenase-mediated oxidation).

S9 fractions from a variety of animal species can be used to predict interspecies differences in the rate of metabolic elimination of the studied compound.

All tests have three replicates per compound and are validated by inclusion of a positive control chemical with known stability under the assay conditions, to check for metabolic competency of the test system. Data provided include a percent remaining compound vs. time diagram, disappearance half-life and/or intrinsic clearance.

# *Experiment summary example*

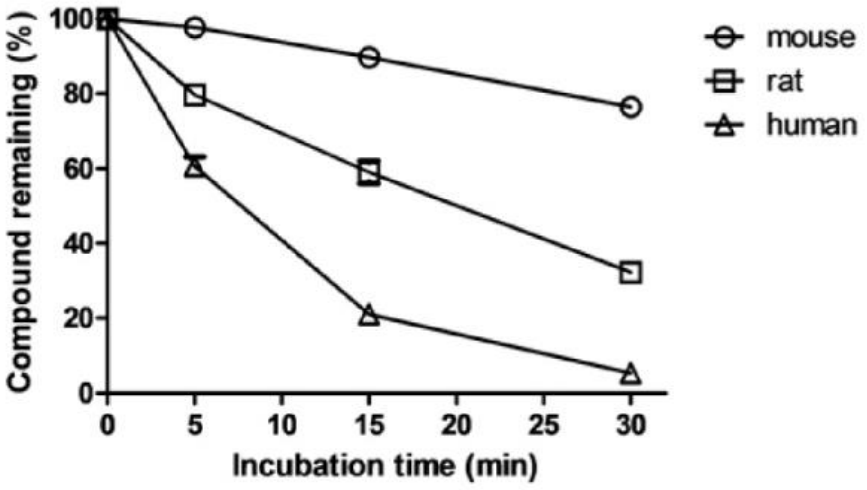


**Fig.1** The diagram shows the time-dependent depletion of an alcoholic group-containing test compound by NADH*-supplemented human, rat and mouse liver S9 fraction. The data are expressed as percentage of compound remaining at each time compared with time 0 min and represent the mean ± SD (n=3). Error bars smaller than the symbols are not visible.

*NADH is a cofactor for both alcohol dehydrogenase- and aldehyde dehydrogenase-dependent oxidations.

**References:**

Parkinson et al. "Biotransformation of xenobiotics in Casarett&Doull's Toxicology: The Basic Science of Poisons" *(*Klaassen CD ed) pp 185–367, McGraw-Hill, Inc., New York, 2013

Lepri et al. "Structure-metabolism relationships in human-AOX: chemical insights from a large database of aza-aromatic and amide compounds." *Proc Natl Acad Sci USA*, 114:E3178-E3187, 2017

## *Protocol:*

| | |
|---|---|
| **Compound requirements** | 50 µL of a 10 mM solution |
| **Test compound concentration** | from 1 to 10 µM, depending on the nature of the analyte |
| **S9 fraction protein concentration** | 1 mg/mL |
| **Cofactors** | NADPH and/or UDPGA (others available on request) |
| **Time points** | 0, 5, 10, 15, 30 minutes (others available on request); longer time points may be needed for a glucuronidation assay |
| **Incubation temperature** | 37°C |
| **Controls** | Incubations of the test compound in the absence of the cofactor(s), as well as in the absence of both cofactor(s) and S9 fraction; incubations of a reference compound with known stability under the assay conditions. |
| **Analysis method** | LC-DAD, LC-UV-fluorescence or LC-MS/MS (depending on the nature of the analyte) |
| **Data delivery** | Percent remaining compound vs. time graph, disappearance half-life and/or intrinsic clearance. |