

# Induced Liver S9 Fraction

## High-Activity Metabolic Activation System for *In Vitro* Genotoxicity Testing

### CONFLICT of INTEREST

All authors are employees and shareholders of MileCell Biotechnology Inc. No other conflicts of interest are declared.

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## BACKGROUND and PURPOSE

Liver S9 fractions are widely used as extrinsic metabolic activation systems in *in vitro* genotoxicity assays to simulate mammalian biotransformation. Co-induction with phenobarbital sodium (PB) and  $\beta$ -naphthoflavone (NF) enhances the expression of major Phase I xenobiotic-metabolizing enzymes, including CYP1A, CYP2B, and CYP3A, thereby expanding the metabolic activation capacity toward indirect mutagens and procarcinogens. This study summarizes the enzymatic characteristics and functional performance of induced liver S9 fractions derived from Sprague-Dawley (SD) rats and LVG Golden hamsters for application in genotoxicity testing.

## METHODS

### S9 Preparation

Liver S9 Fractions were prepared from SD rats and LVG Golden hamsters induced with PB and NF.

### Enzyme Activity Characterization

Cytochrome P450 enzyme activities (CYP1A, CYP2B, CYP3A) were determined and expressed as pmol/min/mg protein.

### Functional Validation

Metabolic activation capacity was evaluated using the Ames bacterial reverse mutation assay and an *in vitro* chromosome aberration assay with indirect mutagens such as 2-aminoanthracene and cyclophosphamide.

## RESULTS

### 1. Representative CYP activity profiles of PB/NF-induced liver S9 fractions

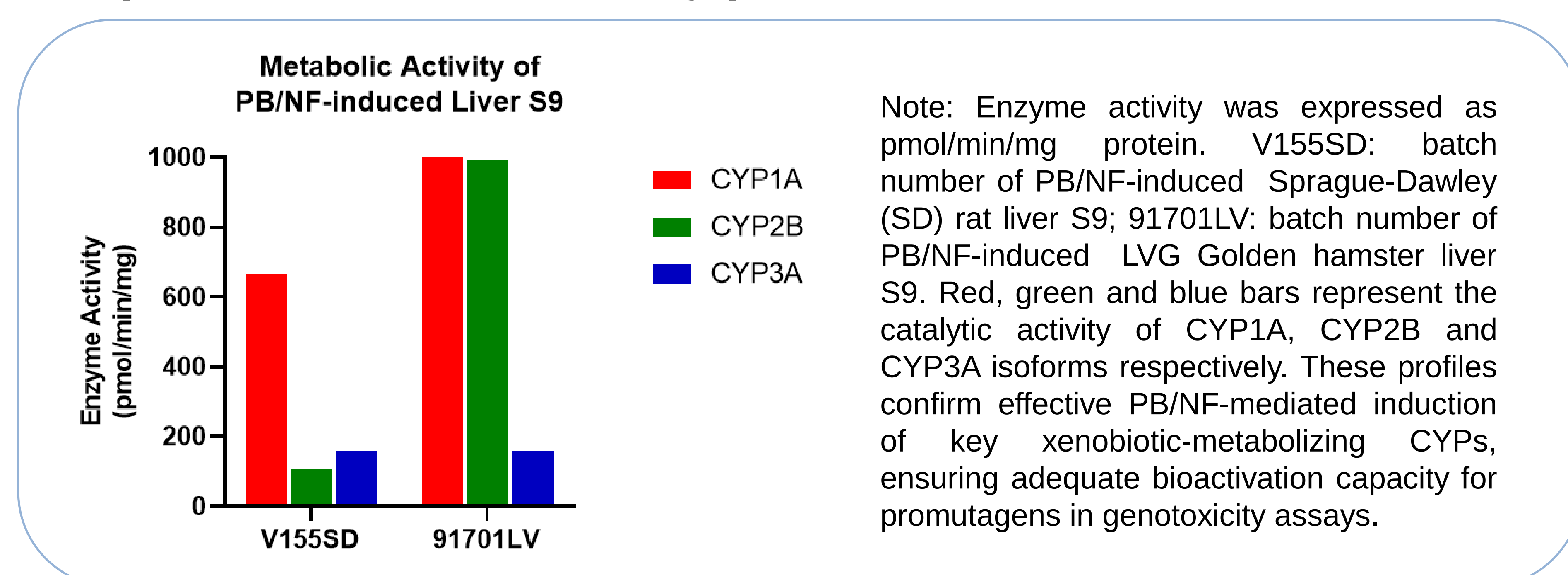


**Fig 1.** Phenobarbital sodium (PB) and  $\beta$ -naphthoflavone (NF)-induced liver S9 fractions were evaluated for CYP-mediated metabolic activity using isoform-selective substrates for CYP1A, CYP2B, and CYP3A. Activities are reported as pmol/min/mg protein. The induced S9 fractions demonstrated strong CYP enzyme activities, supporting their application as metabolic activation systems in Ames, micronucleus, chromosome aberration, and other *in vitro* genotoxicity assays.

### 2. Functional validation of PB/NF-induced liver S9 fractions in metabolic activation-dependent Ames assays

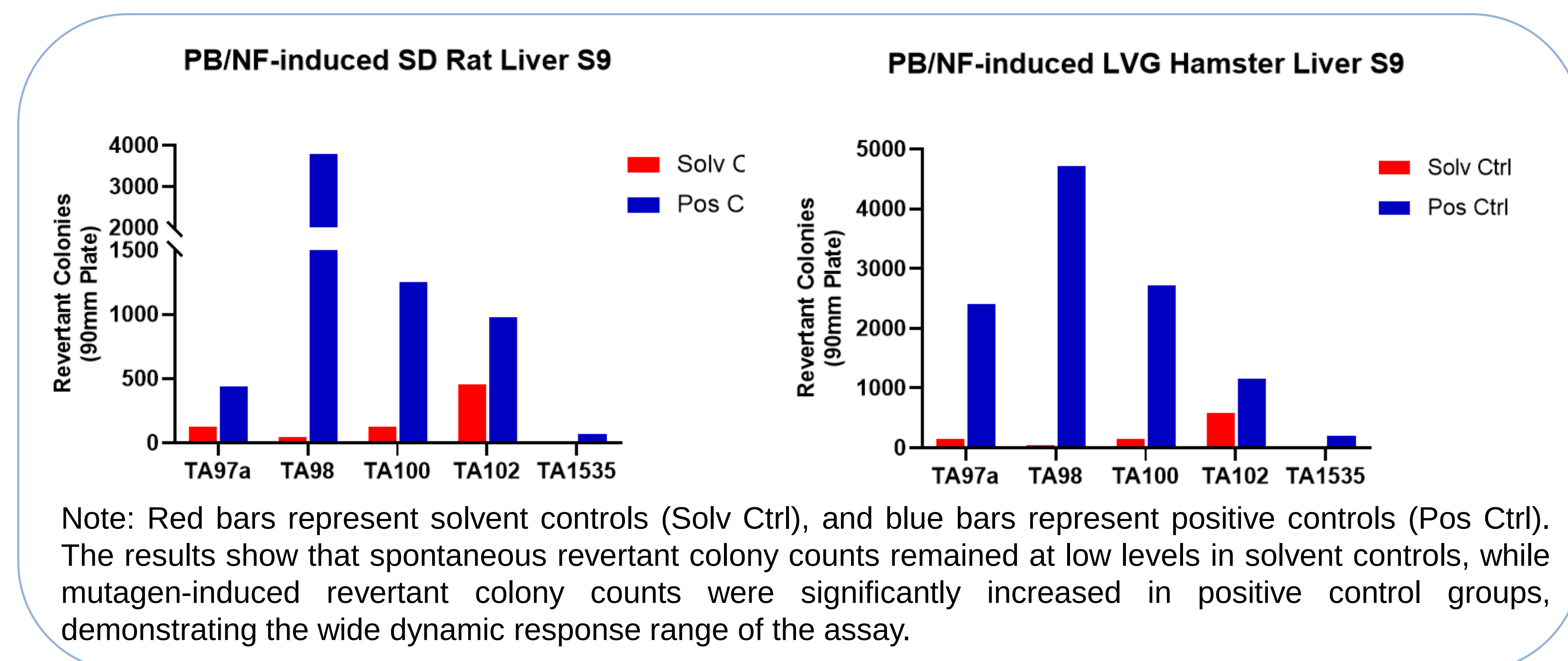


**Fig 2.** PB/NF-induced Sprague-Dawley (SD) rat and LVG Golden hamster liver S9 fractions were validated in Ames assays using five *Salmonella typhimurium* tester strains (TA97a, TA98, TA100, TA102, TA1535). In the presence of S9 metabolic activation, strain-specific positive control compounds induced substantial increases in revertant colony numbers relative to solvent controls, indicating efficient conversion of promutagens to reactive mutagenic metabolites. The results confirm the robust metabolic activation capability of both induced S9 preparations and support their application in regulatory and research-based genotoxicity assessments.

### 3. Functional assessment of induced liver S9 in chromosome aberration assays

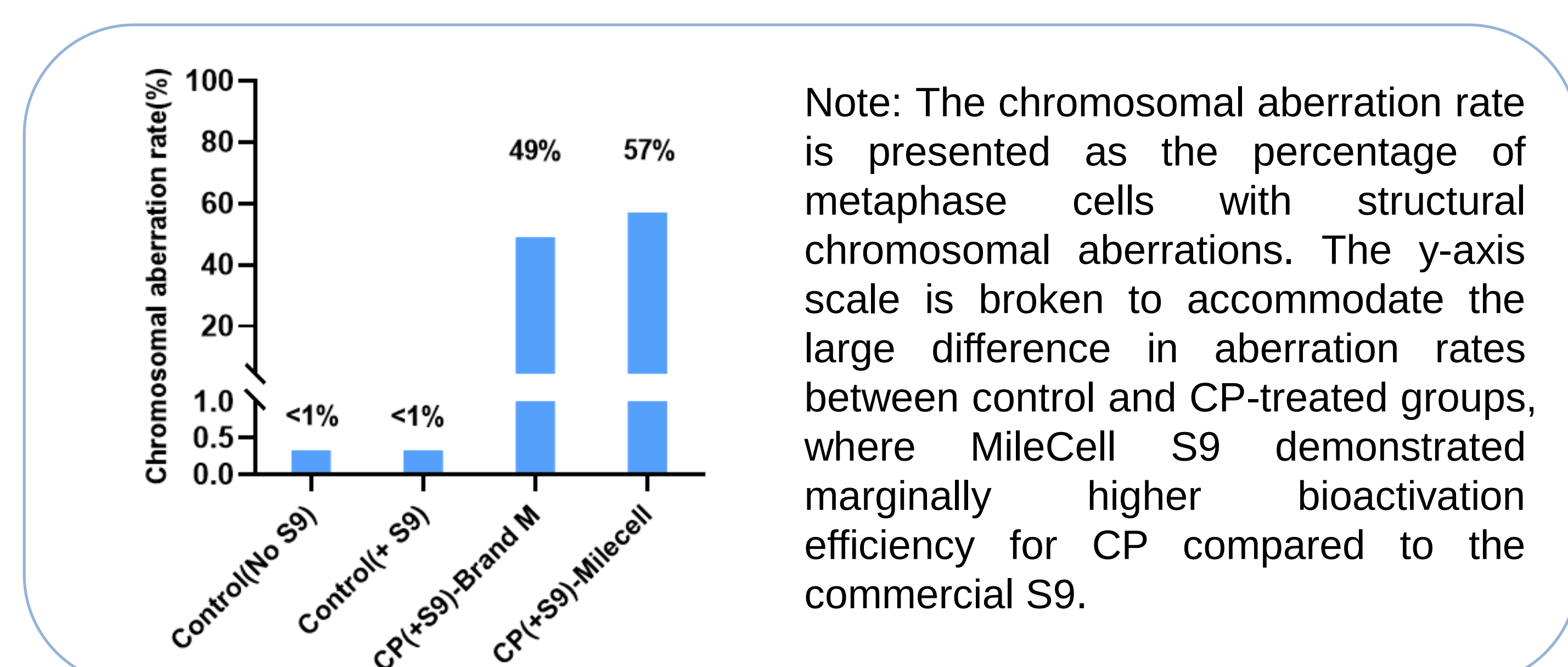


**Fig 3.** To verify the metabolic competence of induced liver S9 fractions, cyclophosphamide (CP) was evaluated in an *in vitro* chromosome aberration assay. As expected, control groups with or without S9 exhibited only background levels of chromosomal aberrations (<1%). Upon metabolic activation by S9, CP induced pronounced chromosomal damage, resulting in aberration frequencies of 49% with a commercial S9 preparation and 57% with MileCell S9. These findings confirm the effective bioactivation capability of MileCell S9 and support its application as a reliable metabolic activation system for *in vitro* genotoxicity testing.

## CONCLUSIONS

PB/NF-induced liver S9 from SD rats and LVG Golden hamsters exhibits high and stable metabolic activity. With comprehensive CYP isoform activities, these S9 preparations are reliable extrinsic activation systems for *in vitro* genotoxicity testing, applicable to drug safety assessment and environmental toxicology research.