

## Regulatory Genotoxicity Test

### Background and Objectives

The development of new drugs requires a thorough investigation of their potential to affect human health before releasing them into the market. This safety evaluation includes assessment of the genotoxic effects in appropriate test systems. The European Union demands genotoxicity data for all new chemical entities (NCE) before the commencement of clinical trials. The International Conference on Harmonization, which brings together the regulatory authorities from the US, Japan, and Europe, issued relevant guidances (S2A, S2B) describing a battery of tests to be conducted by sponsors. Among them, the micronucleus test in peripheral blood of treated mice is employed by Pharmacelsus and represents an acceptable assay for generating reproducible genotoxicity data.

### Experimental Design

At Pharmacelsus, we use the gTox Flow Kit (Beckman Coulter) to quantitate micronucleated polychromatic erythrocytes in male CD1 mice, an accepted endpoint for genotoxicity. This approach enables us to also consider relevant factors such as absorption, distribution, metabolism and excretion, factors not covered by in vitro assays. Pharmacelsus uses a state-of-the-art two-laser, five-color Cytomics FC500 MPL flow cytometer (11 CFR part 11 compliant).



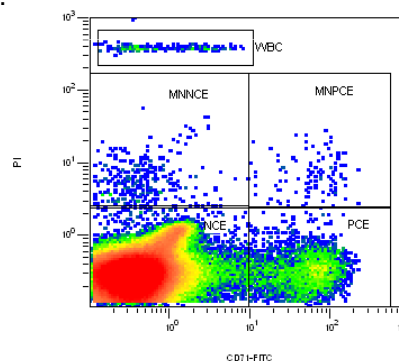
Cytomics FC500 MPL (Beckman Coulter)

### Data Acquisition and Analysis

According to regulatory requirements, male CD1 mice will be exposed systemically to the test compound. After 36-48 hours, heparinized blood will be collected and whole blood will be processed using the gTox flow kit. At least 2,000 reticulocytes will be collected for each individual mouse. Data will be presented according to international standards as % mononucleated reticulocytes. Samples will be compared with untreated mice and mice treated with mitomycin C or cyclophosphamide.

### Quality Benchmarks and Controls

According to regulatory guidelines, mice treated with mitomycin C or cyclophosphamide will be used as positive controls for unmetabolized or metabolized compounds, respectively. The performance of the flow cytometer will be monitored by measuring predetermined parameters using standardized beads.



Detection of micronucleated reticulocytes (upper right quadrant) within peripheral blood.

### Supplemental Testing

In addition to genotoxicity tests, other parameters such as membrane integrity, reactive oxygen species and cell cycle distribution can be added to the test battery to obtain additional data with toxicological meaning.

*Please don't hesitate to contact us for a customized quotation*

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