

Acute Toxicity Screen

For rapid and accurate evaluation of acute toxicity.

This very affordable screen requires less than 2 mg of compound and provides data in less than 2 weeks.



Problem: High-throughput screening platforms designed to identify new chemical entities (NCEs) with potential therapeutic value, yield several possible "Hits". Discovery teams are then faced with the task of identifying a handful of promising NCEs for further discovery research; often referred to as the Lead Optimization phase. Traditionally, this phase has focused on the desired or "drugable" properties of a potential candidate before understanding potential compound liabilities associated with toxicity. In order to reduce development costs, it is essential that information on the relative safety of new compounds be evaluated early. Single endpoint toxicity assays with a single exposure concentration will not provide adequate information for selecting compounds. However, at this early stage in development, company resources applied to compound development must be used wisely. Toxicity data must be robust enough to allow teams to rank-order new "Hits" for a large number of compounds, based on *in vivo* relevance, the data must be obtained in a short period of time, and at a reasonable cost.

Solution: CeeTox scientists have many years of experience in pharmaceutical drug discovery. This knowledge has been used to develop and validate *in vitro* screening models for assessing toxicity. State-of-the-Art robotics platforms enable us to test a large number of compounds, and provide consistent and reproducible data. Our goal is to provide reliable information that can be used with confidence by our customers during the process of selecting the best "Hits" for further development. The Acute Toxicity Screen was designed after evaluating hundreds of different drugs and chemicals. This cell-based system accurately identifies compounds that would produce acute toxicity in a rat 14-day repeat-dose study. Membrane integrity and mitochondrial function are monitored at 3 exposure concentrations in 4 replicates over a 24-hr period.

The Importance of Multiple Endpoints and Dose-Response: Many organizations make the mistake of using a single endpoint at a single exposure concentration. When this testing design is applied to early toxicity screening the probability of false positive or false negative results is high. When 2 or more endpoints are monitored over several exposure concentrations, however, the data obtained are considerably more useful.

As shown in the example in the chart below the rodenticide rotenone was a potent inhibitor of mitochondrial function with a half maximal response of approximately 8 μM . In comparison, cell death as measured by membrane integrity, had a delayed response with a half maximal effect estimated to be 45 μM . Clearly, the risk associated with this compound is related to the disruption of mitochondrial function, which then leads to cell death.

Analysis of mitochondrial function without membrane integrity would not provide any information on the effect and timing of mitochondrial impairment or on the significance of this subcellular target. A focus on membrane integrity would underestimate the toxicity of the compound. When the 2 endpoints are combined over several exposure concentrations, however, a clearer profile can be seen. The compound is a potent inhibitor of mitochondrial function, and this is the primary target. Under the conditions tested, cell death was delayed, as evidenced by the higher concentration required to produce a half maximal effect (TC50).

State-of-the-Art Laboratories and Equipment: CeeTox robotics platforms can analyze more than 50 compounds per week, delivering toxicity evaluations to discovery teams in approximately 2 weeks. New hits can be analyzed based on a ratio of the IC50 data for target interaction (potency/efficacy) to the toxicity (Ctox) value. The resultant *in vitro* therapeutic index allows compounds to be rank-ordered based on desired drug attributes as well as toxicity.

The CeeTox Difference: The biochemical endpoints, exposure concentrations, and exposure times were selected based on many years of *in vitro* evaluations of new drug candidates. Our proprietary process of analyzing the dose-response data, combined with our internal database, enables CeeTox to provide an accurate assessment of compound toxicity. We can customize assays and the cell type used to address specific customer needs. CeeTox provides its customers with more than a table of numbers representing live and dead cells. The data is predictive and linked to *in vivo* parameters. CeeTox scientists are problem solvers, and as such, we think of ourselves as an extension of our customers' research.

Rotenone Data Sets

