ABUSE LIABILITY ASSESSMENT IN RATS OR NON-HUMAN PRIMATES:
DRUG DISCRIMINATION

STUDY DESIGN:
Species: Monkey (Cynomolgus or Rhesus) and/or Rats
Total animals: 6 monkeys and/or 20 rats

<table>
<thead>
<tr>
<th>Drug Dose vs. Vehicle</th>
<th>Male Rats Only</th>
<th>Male Monkeys Only</th>
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</thead>
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<td></td>
<td>20</td>
<td>6</td>
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DOSING: The test article will be administered by subcutaneous, intramuscular, or intraperitoneal injections, or oral gavage. Intravenous dosing of the training drug in a drug discrimination study is not recommended due to the length and complexity of the procedure and the patency life of the catheter placements in rats. Behaviorally active doses may be determined based on the outcome of a separate assessment of schedule controlled behavior (see supplemental outline, below).

GENERAL PROCEDURES:
A group of 20 animals will be trained to discriminate the presence and absence of a dose of a standard positive control test article (i.e., morphine, cocaine, chlordiazepoxide, etc) in a two choice (drug vs vehicle) food-reinforced drug discrimination task under a fixed ratio 10 (FR10) schedule in daily training sessions. Training sessions will last for 15 minutes or until the delivery of 50 reinforcers, whichever occurs first.

Daily drug training sessions will be alternated with vehicle training sessions in a pseudo-random order until the rats demonstrate greater than 80% stimulus-appropriate lever press responding and less than 18 responses prior to the delivery of the first reinforcer (FRF). In general, animals will be trained five days per week. Each animal will be required to meet these criteria through a double alternation sequence prior to the commencement of test sessions (i.e., Drug, Drug, Vehicle, Vehicle).

Once the criteria for stimulus control have been demonstrated for the double alternation sequence, each animal will be tested with either vehicle and/or various doses of the training drug (usually 4 doses in a pseudo-random order). During test sessions, ten consecutive responses on either lever will be reinforced. Test sessions will be alternated with training sessions to assure the accuracy of the discrimination during the testing phase of the study. If stimulus control is not demonstrated during a training session, further testing will be halted until each animal demonstrates stimulus control at the >80% accuracy criterion.

Each selected dose of the test and control articles will be tested in 6 to 10 trained rats, or 4-6 monkeys. Since training sessions are alternated with test sessions, test sessions will be considered an independent event for analysis. Once the complete dose-response curve for the training drug is established, a dose-response function of the test article of interest will be determined over successive test-training sessions.

Complete generalization of the test article to the training dose of the training drug will be considered to have been demonstrated if >80% of the total session responses are emitted on the drug-appropriate lever during free-choice test sessions with the test article. Response rates will provide a second measure of behavioral activity/toxicity of the compound of interest.

DEPENDENT MEASURES: The percentage of the total session responses emitted on the drug-appropriate lever (% drug-appropriate responding) will be assessed along with rates-of-responding, expressed as a percentage of vehicle control rates, over 5 vehicle training sessions.
Conduct ABUSE LIABILITY ASSESSMENT IN RATS OR NON-HUMAN PRIMATES: DRUG DISCRIMINATION

conducted over the course of the dose-response function (resp/sec). The average number of
responses emitted prior to the delivery of the first reinforcer (FRF) will also be monitored and
reported.

CLINICAL OBSERVATIONS: Predose and following the completion of the drug
discrimination training/test session

BODY WEIGHTS: Prior to each dose

STATISTICAL ANALYSIS: Standard parametric statistical analyses

ANALYTICAL CHEMISTRY: Standard sample collections performed to support dose
formulation methods (analysis at an additional cost).

FINAL REPORT: Standard GLP compliant report for regulatory submission
ABUSE LIABILITY ASSESSMENT IN RATS OR NON-HUMAN PRIMATES:
DRUG DISCRIMINATION

SCHEDULE-CONTROLLED OPERANT BEHAVIOR IN THE RAT

STUDY DESIGN:
Species: Monkey (Cynomolgus or Rhesus) and/or Rats
Total animals: 6 monkeys and/or 20 rats

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<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>20</td>
<td>6</td>
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TRAINING: All animals will be initially maintained at 80% of free-feeding body weights. Each rat will be trained in standard operant chambers to respond on a lever for food reinforcement under a fixed-ratio 30 schedule of food delivery. Animals will be trained until each demonstrates less than 10% variability across five consecutive training sessions. Sessions will be 40 minutes in duration; there will be no limit to the number of reinforcer deliveries during the training sessions.

DOsing: The test article will be administered by the desired route.

TESTING: Rats will be tested with vehicle and three selected doses of the test article. The doses will be selected along a logarithmic scale to generate functional four point dose-effect function for the rate-suppressant effects of the test article. Vehicle will be tested in each of the animals that achieve stable rates-of-responding in the operant task. Test article will be tested in 10 rats randomly selected from the pool of possibly 20 trained rats. Each test session will be separated by at least one week of training to allow for a washout period and to demonstrate regaining of stable responding following dosing. Each test session will be considered an independent event in an industry standard repeated measures analysis of variance.

DEPENDENT MEASURES: The raw rates-of-responding will be expressed as responses-per-second and as a percentage of vehicle control rates.

CLINICAL OBSERVATIONS: Predose and following the completion of the test article test session

BODY WEIGHTS: Prior to each dose

STATISTICAL ANALYSIS: Standard parametric statistical analyses

ANALYTICAL CHEMISTRY: Standard sample collections performed to support dose formulation methods (analysis at an additional cost).

FINAL REPORT: Standard GLP compliant report for regulatory submission

To receive information about this study, please contact Dr. Ted Baird at Ted.Baird@mpiresearch.com or call 1-269-668-3336.