ABUSE LIABILITY ASSESSMENT IN RATS AND/OR NON-HUMAN PRIMATES: SELF ADMINISTRATION

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>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

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
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 AND/OR NON-HUMAN PRIMATES: SELF ADMINISTRATION

STUDY DESIGN:

Species: Monkey (Cynomolgus or Rhesus) and/or Rats

Total animals: 6-12 monkeys and/or 20 rats

<table>
<thead>
<tr>
<th>Training Drug</th>
<th>Male Rats Only</th>
<th>Male Monkeys Only</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mg/kg/injection cocaine hydrochloride</td>
<td>24</td>
<td>6-12</td>
</tr>
</tbody>
</table>

DOSING: The test article will be administered through chronic indwelling venous catheters, contingent upon a schedule of reinforcement, as indicated below. Behaviorally active doses may be determined based on the outcome of a separate assessment of schedule controlled behavior (see supplemental outline, below).

GENERAL PROCEDURES: Animals will be trained to press a lever for the delivery of a standard food pellet. Over successive training sessions, the animals will be trained to emit 5 responses for each food pellet delivery (FR5). Once animals demonstrate stable performance at the FR5 schedule of food maintained lever press responding, catheter placement surgery will be implemented. Following a brief post-surgical recovery period, animals will be placed back into the operant chambers and allowed to respond for the simultaneous delivery of food pellets and cocaine on an FR5 schedule. For this single session, only, animals will respond for the delivery of a food pellet and the simultaneous delivery of a 0.5 mg/kg infusion of cocaine through the venous catheter. Following this induction procedure, the food pellet dispenser will not operate, and the animal will respond for the delivery of a single bolus injection of 0.5 mg/kg maintenance dose of cocaine. Adjustments to the schedules of reinforcement or training stimuli are possible, based on the species employed on study.

Using cocaine as the initial training stimulus and then transferring to morphine has been demonstrated to take less time than attempting to train self-administration of opiates from the start of training. Cocaine is usually self-administered within the first training session. Once animals are trained to cue into the subjective and reinforcing properties of cocaine, it is easier to transfer over to other exemplars of drug abuse (opiate, benzodiazepine, etc).

Daily sessions will begin with a small (approx 40µL) “priming” injection of cocaine at the beginning of the session to flood the catheter with the drug so that it is immediately available to the animal following the first response ratio requirement of the session.

Animals will be trained in daily sessions to receive a bolus of 0.5 mg/kg cocaine until each animal demonstrates stable drug-maintained responding, defined as less than ±15% variability in reinforcer deliveries per 1 hour sessions occurred.

Once self-administration has been demonstrated with cocaine, substitution procedures will be implemented to switch over to the maintenance dose of the selected reference drug. Once stable performance is achieved with the reference drug, a dose response curve will be generated by substituting a randomly selected test dose of the drug for the training dose in 6 (rats) or 4-6 (monkeys) animals per dose.

Dose response curves will be generated for the maintenance drug in test sessions, usually conducted on Fridays (i.e., following several days of consistent responding). The selected doses of cocaine will span a behaviorally active range that has been shown to produce “extinguished”
ABUSE LIABILITY ASSESSMENT IN RATS AND/OR NON-HUMAN PRIMATES:
SELF ADMINISTRATION

responding at the low end of the dose range, maximal rates-of-responding at the intermediate
doses, and response rate suppression at the high end of the dose range.

SUBSTITUTION TESTS: Selected doses of the test article will be substituted for the usual
dose of the maintenance drug during weekly substitution test sessions. A range of at least 4 doses
of test article and its control vehicle will be substituted for the maintenance drug in individual test
sessions conducted in 4-6 animals. Substitution tests will be conducted for at least three
consecutive days. Substitution for the maintenance drug by the test article will be defined as a
pattern of responding characterized by less than 15% variability, while exhibiting a sustained
pattern of reinforced behavior, across 3 consecutive days of substitution.

Each selected dose of the test and control articles will be tested in 4-6 trained animals. Since
training sessions are alternated with test sessions, each test session will be considered an
independent event for analysis.

DEPENDENT MEASURES: The total number of injections self administered by the animal
during the session, the total drug dose delivered, as well as the rates-of-responding will be
reported. Dose response functions will be generated for all three measures.

CLINICAL OBSERVATIONS: Predose and following the completion of the drug
self-administration 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 AND/OR NON-HUMAN PRIMATES:
SELF ADMINISTRATION

SCHEDULE-CONTROLLED OPERANT BEHAVIOR IN THE RAT

STUDY DESIGN:

Males Only

Group 1 20

TRAINING: All rats 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 rat 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 a 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@mpiireseach.com or call 1-269-668-3336.