PROTOCOL #: NIDA-MDS-BupropionMeth-0001

PHASE 2, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL OF BUPROPION FOR METHAMPHETAMINE DEPENDENCE

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1 LIST OF ABBREVIATIONS

Abbreviation	Definition
ACDS	Adult ADHD Clinical Diagnostic Scale
ADD	Attention Deficit Disorder
ADHD	Attention Deficit Hyperactivity Disorder
AE	Adverse Event
AISRS	Adult ADHD Investigator Symptom Rating Scale
ALP	Alkaline Phosphatase
ALT/SGPT	Alanine Aminotransferase/Serum Glutamic Pyruvic Transaminase
ASI-Lite	Addiction Severity Index-Lite
AST/SGOT	Aspartate Aminotransferase/Serum Glutamic Oxaloacetic Transaminase
BDNF	Brain-derived neurotrophic factor
BE	Benzoylecgonine
BSCS	Brief Substance Craving Scale
BUN	Blood Urea Nitrogen
CAP	College of American Pathologists
CBT	Cognitive Behavioral Therapy
CGI-O	Clinical Global Impression Scale – Observer
CGI-S	Clinical Global Impression Scale – Self
CLIA	Clinical Laboratory Improvement Amendment of 1988
COMT	Catechol-O-methyltransferase
CPK	Creatinine phosphokinase
CYP2D6	Cytochrome P450 2D6
Del	Deletion
dL	Deciliter
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders Fourth Edition
DTR&D	Division of Treatment Research and Development
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EST	Expressed sequence tags
GABRG2	Gamma-aminobutyric acid receptor gamma 2 subunit gene
GGT	Gamma glutamyltranspeptidase
HAM-D	Hamilton – Depression Rating Scale
HIV	Human immunodeficiency virus
HRBS	HIV Risk-Taking Behavior Scale
HWE	Hardy-Weinberg Equilibrium
Ins	Insertion
kg	Kilogram
LAAM	Levomethadyl acetate (L-alpha acetylmethadol)
LDH	Lactate dehydrogenase
LD	Linkage disequilibrium
mg	Milligrams
mL	Milliliter
MAO	Monoamine oxidase

Abbreviation	Definition
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MINI	Mini International Neuropsychiatric Interview DSM-IV version
NIDA	National Institute on Drug Abuse
NTS	Nicotine transdermal system
OTC	Over-the-counter
ROS	Reactive oxygenation species
RPR	Rapid plasma reagin (test for syphilis)
SAE	Serious adverse event
SNPs	Single nucleotide polymorphisms
SR	Sustained-release
SSRI	Selective serotonin reuptake inhibitor
SUR	Substance use report
V_{ss}/F	Volume of distribution
VMAT-2	Vesicular monoamine transporter-2

2 STUDY SCHEMA

Study Week	Activity		Bupropion 300 mg/day N = 100
-4	Screening/ Baseline Assessments (weeks -4 to 0)		
0	Randomization Double-blind Investigational products* & assessments (weeks 0 to 12)	**Strata** •Methamphetamine use •Clinical Site •Severity of depression •Adult ADHD	Placebo N = 100
12			
16	Final follow-up at week 16		
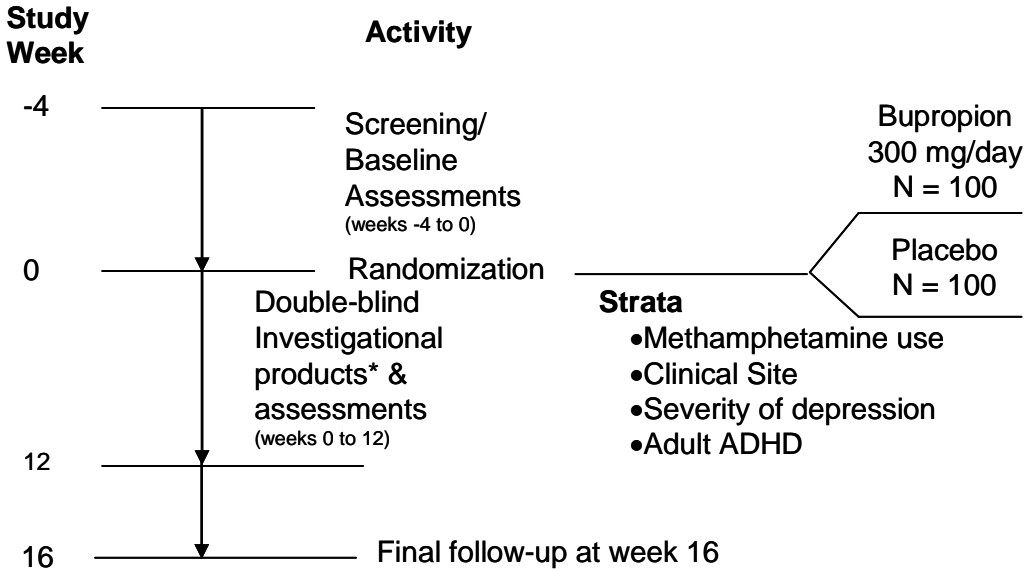
> *Double-blind investigational products consists of daily bupropion (Days 1 – 3: 150 mg/day, Days 4 – 81: 300 mg/day, Days 82 – 84: 150 mg/day) or matching placebo. All get thrice-weekly, group cognitive behavioral therapy.

3 PROTOCOL SYNOPSIS

STUDY OBJECTIVES: The primary objective of this study is to assess the efficacy of bupropion in reducing methamphetamine use in subjects with methamphetamine dependence who report using methamphetamine 29 or less days during the 30 days prior to signing consent. It is hypothesized that bupropion, compared to placebo, will be associated with an increase in the proportion of subjects who achieve abstinence (confirmed by at least two methamphetamine-negative urines and no methamphetamine-positive urines) each week during the last two weeks (Weeks 11 and 12) for non-daily users. Secondary objectives include but are not limited to: assessing the success or failure to achieve abstinence (confirmed by at least two methamphetamine-negative urines and no methamphetamine-positive urines) each week during the last two weeks (Weeks 11 and 12) for subjects using methamphetamine 18 or less days during the 30 days prior to signing consent, assessing the safety of bupropion in this study population, assessing other measures of efficacy of bupropion in reducing methamphetamine use or craving, and other psychological assessments of methamphetamine dependence. In addition, if efficacy findings are promising, preliminary evaluations of the genetic markers associated with a positive clinical outcome or predictive of bupropion safety and efficacy including associations with bupropion pharmacokinetics (PK) may be performed.

STUDY DESIGN: This is a double-blind, placebo-controlled, parallel-group design study in which, after an up to 4-week screening period which includes a 2-week baseline assessment period, 200 subjects will be randomly assigned in an approximately 1:1 ratio to either receive placebo or bupropion (100 subjects per group) daily for 12 weeks, with follow-up assessments weekly for 4 weeks after completion of study interventions. Adaptive randomization will be used to balance study intervention groups within each clinical site, based on methamphetamine use (18 or less days *versus* 19-29 days out of 30 days prior to signing consent), severity of depression symptoms [Hamilton Depression Rating Scale (HAM-D) score ≤ 12 *versus* >12], and presence of adult Attention Deficit/Hyperactivity Disorder (Adult ADHD Clinical Diagnostic Scale - ACDS).

STUDY POPULATION: Two hundred treatment-seeking individuals with Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) criteria for methamphetamine dependence determined by Mini International Neuropsychiatric Interview (MINI) DSM-IV version will be randomized to receive either bupropion or placebo. Only those subjects who report 29 or less days of methamphetamine use in the 30 days prior to signing consent will be considered for enrollment. Males and females, 18 to 65 years-of-age, inclusive, with at least one methamphetamine-positive urine specimen provided during the screening or baseline period prior to randomization (or provide collateral information to verify recent use if a positive urine sample can not be obtained), who verbalize the ability to understand and provide written informed consent, will be included.

INTERVENTIONS: Subjects randomized to the bupropion group will receive 150 mg of sustained-release (SR) bupropion once daily for 3 days then twice daily, i.e., a total of 300 mg/day, for a total of 12 weeks (dose taper will be performed the last 3 days of the 12 weeks, to 150 mg per day). Subjects randomized to the placebo group will receive matched placebo tablets according to the same schedule. All subjects will receive two 60 minute early recovery group

sessions after signing consent but before randomization, and manual-guided group cognitive behavioral therapy (CBT) consisting of three 90 minute sessions/week during the 12 weeks of study interventions and once weekly during the 4 weeks of follow-up.

SAFETY ASSESSMENTS: All subjects enrolled in the study will have a physical examination, a 12-lead electrocardiogram (ECG), and clinical laboratory studies (blood chemistry, hematology, Hepatitis B and C virus serology, syphilis test, tuberculin test, and urinalysis) performed during screening. If the potential participant is female, a pregnancy test will also be performed during screening, on the first day of investigational product administration, at Study Weeks 4, 8, and 12 or earlier termination. Vital signs, concomitant medication use, and a urine screen for other substances of abuse will be assessed weekly during the study. Adverse events (AEs) will be assessed at each visit. At the end of the study, or if terminated early, subjects will be evaluated for AEs, vital signs, physical examination, clinical laboratory studies, and ECG.

EFFICACY ASSESSMENTS: The primary efficacy outcome measure is a binary measurement of treatment success or failure, where a subject who successfully achieves two weeks of abstinence during the last two weeks of investigational product dosing (Weeks 11 and 12) is scored as a success. A successful outcome requires that (1) all urine samples tested in the last two weeks are negative for methamphetamine (< 300 ng/mL quantitative result of negative qualitative result) and (2) a minimum of two urines samples are collected and tested per week during Weeks 11 and 12. The study population for the primary efficacy outcome measure includes non-daily users defined as subjects with methamphetamine dependence who reported using methamphetamine 29 or less days during the 30 days prior to signing consent. A subject who drops out before the last 2 weeks of the intervention phase is scored as a failure in the analysis. A subject who stays on study to the beginning of Week 11 but who does not provide at least 2 urine samples per week during Weeks 11 and 12 is scored as a failure. Secondary assessments include analyses of other measures of success in the reduction of methamphetamine use, including but not limited to success or failure to achieve abstinence (confirmed by at least two methamphetamine-negative urines and no methamphetamine-positive urines) each week during the last two weeks (Weeks 11 and 12) for subjects using 18 or less days during the 30 days prior to signing consent, the $\log_{10}$ weekly mean quantitative urine methamphetamine level, the maximum number of days of methamphetamine negative urines, the proportion of successful subjects with different patterns in the reduction in methamphetamine use, the weekly mean proportion and the maximum number of consecutive non-use days by self report, and proportion of subjects who achieve abstinence who remain abstinent through the end of follow-up (Week 16). Additional measures of bupropion effect will include study retention, Addiction Severity Index (ASI)-Lite scores, HAM-D scores, Adult ADHD Investigator Symptom Rating Scale (AISRS) scores, Brief Substance Craving Scale (BSCS) scores, Clinical Global Impression scores as assessed by the subject (CGI-S) and an observer (CGI-O), and the HIV Risk-Taking Behavior Scale (HRBS) score. ASI-Lite and the HRBS are performed during baseline and at study termination. The HAM-D is performed weekly during screening/baseline, every other week during the intervention period, and also once at study termination. The BSCS, CGI-S, and CGI-O are assessed weekly during screening, baseline, and intervention periods, and at study termination. AISRS is measured at baseline, and Weeks 4, 8, and 12, or early termination. Investigational product compliance will be monitored by counting returned investigational product tablets and by measuring bupropion and its major metabolite in urine samples. Effects on

other substances of abuse (nicotine, alcohol, and marijuana) will be determined by self-report collected at each visit.

GENETICS EVALUATION: For subjects who consent to the genetics part of the study, blood will be collected before the start of investigational product administration for analysis of genetic variants and if the subject is randomized into the study, during Study Weeks 6 and 12 for analysis of gene expression profiles during interventions to evaluate genetic variants and genes associated with positive clinical outcomes and safety. The Affymetric 500K Array set will be used to identify susceptibility genes for methamphetamine dependence, with the special emphasize on those genes involved in the dopaminergic reward system as well as gene expression profiles of subjects in response to bupropion over different time points. Conduct of these analyses will be dependent upon favorable efficacy findings.

Blood will be collected before investigational product administration and during Study Weeks 6 and 12 for bupropion levels and plasma brain derived neurotrophic factor (BDNF) levels, to examine associations between genetic variants expression levels, bupropion pharmacokinetics (PK), plasma BDNF levels, and clinical response. Elevated plasma BDNF levels are associated with methamphetamine neurotoxicity, and are known to decline during abstinence from methamphetamine. Since bupropion also has an independent effect on BDNF, plasma samples will be tested for bupropion at the same timepoints as plasma for BDNF is obtained. This will allow the evaluation of whether BDNF is affected differently in subjects receiving bupropion compared to those receiving placebo, and whether this effect influences methamphetamine use or abstinence. In addition, it is also hypothesized that methamphetamine users who have the BDNF 66Val homozygote will be more likely to benefit from bupropion than those who do not.

ANALYSIS: Each of the primary and secondary outcome variables will be analyzed using appropriate statistical methods for the intention-to-treat (ITT) population and for the evaluable population. The ITT population includes subjects who are randomized and receive at least 1 dose of investigational product. The evaluable population is those subjects who meet the eligibility criteria, receive at least 4 weeks of investigational product, and provide at least 2 urine samples per week that were analyzed for methamphetamine during the last two weeks of investigational product dosing period (Weeks 11 and 12). The proportions of subjects who achieve abstinence during Weeks 11 and 12 will be compared between groups using either the Chi-square test or Fisher exact test, as appropriate based on the estimated expectation of the contingency table's cell sizes computed from the table's row and column totals. The effect, if any, of age, race, usual route of methamphetamine use (oral/nasal inhalation *versus* intravenous/smoked), or clinical site on the primary intervention effect will be determined. Interactions of intervention group with methamphetamine use (18 or less days *versus* 19-29 days out of 30 days prior to signing consent), severity of depression (HAM-D score ≤ 12 *versus* >12), and presence of adult ADHD will also be assessed. Statistical tests will be two-sided at a 5% Type I error rate. Ninety-five percent confidence intervals will be two-sided. In addition, all of the above-mentioned endpoints will be compared in a medication-compliant subset of the evaluable bupropion group, a medication non-compliant subset of the evaluable bupropion group, and the evaluable placebo group. Medication-compliant subjects are defined as individuals testing positive for bupropion and/or its major metabolite in at least 50% of urine samples (in the aggregate) collected during

study weeks 1-10 and in at least 66% of urine samples (in the aggregate) collected during weeks 11 and 12.

Summaries of the baseline characteristics of the subject population in both intervention groups will be prepared, for both the ITT and evaluable subject populations. A summary will be prepared to show dropouts/retention over time in each intervention group and for major subgroups. Investigational product compliance of each group will be summarized.

All AEs will be reported in tabular form, indicating the frequency of each type of event by study intervention group, and by the following demographic characteristics such as gender, ethnicity, age, duration of addiction, and all combinations of these characteristics.

Association analysis for either single or multiple single nucleotide polymorphisms (SNPs) (as a block) from GeneChip® Mapping 500K Array will be conducted by using the ALLELLE, CASECONTROL and HAPLOTYPE procedures of SAS/Genetics. CASECONTROL will also be used to compare allele and genotype frequencies in the bupropion and placebo groups using three types of Chi-square tests (i.e., genotype and allele case-control tests and linear trend test) and options for controlling correlation of allele frequencies among members of the same subpopulation. Similarly, we plan to test association using haplotype information from multiple SNPs within a candidate gene/region for the case-control data. In addition, for the bupropion group, we also plan to examine the association between genetic markers and trough plasma levels of bupropion and safety outcomes such as frequency of severity of commonly reported AEs (e.g., insomnia). Furthermore, we plan to analyze interactions between significant genes or SNPs and environmental factors using a generalized multifactor dimensionality reduction method. Conduct of these assays will be dependent upon favorable efficacy findings.

If differentially expressed genes are identified, we plan to use various clustering techniques over different experimental groups to analyze the expression patterns. Expression profiles will be grouped by: a) hierarchical cluster analysis using CLUSTER and TREEVIEW b) quality clustering and c) self-organizing maps. The outputs from each program will be compared with respect to the clusters formed. These data will be used to determine relationships between the clusters, disease status, and intervention group and response.

4 INTRODUCTION

4.1 Methamphetamine

Methamphetamine (Methedrine, "speed", "ice", "meth", "crank") is used and misused as a central nervous system stimulant. Methamphetamine (N-methylamphetamine) is a non-cathecholamine phenylisopropanolamine that belongs to the ephedrine family of sympathomimetic drugs. The drug is made easily in clandestine laboratories with relatively inexpensive over-the-counter ingredients. These factors combine to make methamphetamine a drug with high potential for widespread abuse.

4.1.1 Pharmacology
Methamphetamine acts primarily by increasing release of stored catecholamines - dopamine, epinephrine, and norepinephrine. It also inhibits monoamine oxidase (MAO), an action that

would increase its cathecholaminergic activity. Amphetamines affect serotonergic systems as well. Thus, *d*-amphetamine releases serotonin and may act as a direct agonist of serotonin receptors [1,2]; it has been shown to increase serotonergic neurotransmission by inducing the firing rate of serotonergic cells in the raphe nucleus.[3] Methamphetamine abusers demonstrate a significantly lower level of dopamine D2 receptors, with a difference of 16% in the caudate and 10% in the putamen compared to non-drug abusing controls as assessed by positron emission tomography (PET) with [^{11}C]-raclopride.[4] This low level of D2 dopamine receptors is associated with a lower level of glucose metabolism in orbitofrontal cortex (assessed by PET with fluorodeoxyglucose) suggesting that D2 receptor-mediated dysregulation of the orbitofrontal cortex could underlie a common mechanism for loss of control and compulsive drug intake in drug addicted subjects.[4]

Methamphetamine readily enters the central nervous system, and has a marked stimulant effect on mood[5] and alertness.[6,7] Methamphetamine is neurotoxic to dopamine terminals when administered to laboratory animals, including monkeys.[8-10] Studies in methamphetamine abusers have also documented significant loss of dopamine transporters (used as markers of the dopamine terminal) that are associated with slower motor function and decreased memory. The extent to which the loss of dopamine transporters predisposes methamphetamine abusers to neurodegenerative disorders such as Parkinsonism is unclear and may depend in part on the degree of recovery. The effects of protracted abstinence on the loss of dopamine transporters in striatum has been studied in methamphetamine abusers using PET and [(^{11}C)]d-threo-methylphenidate (dopamine transporter radioligand).[11] Brain dopamine transporters in five methamphetamine abusers evaluated during short abstinence (<6 months) and then retested during protracted abstinence (12-17 months) showed significant increases with protracted abstinence (a difference of 19% in the caudate and 16% in the putamen) that was accompanied by increase in thalamic, but not striatal, glucose metabolism (assessed by PET with fluorodeoxyglucose); however, although the performance in motor and verbal memory tests showed some improvement, this effect was not significant.[11,12] These data indicate that dopamine terminals can either recover during protracted abstinence or that remaining viable terminals increase arborization, but it is not sufficient for complete function recovery as there was no improvement in cognitive tests.[11] These findings have treatment implications because they suggest that protracted abstinence may reverse some of the methamphetamine-induced alterations in brain dopamine terminals, but other deficits persist.

Other studies confirm that methamphetamine use may result in a long-term damage to neurons involved in cognitive function. Thus, brain imaging studies (magnetic resonance spectroscopy) show neuronal damage in basal ganglia and frontal white matter with a concomitant increase in size/number of glial cells in subjects with a history of methamphetamine abuse that have been abstinent for as long as 21 months.[13] The PET studies revealed glucose metabolism abnormalities in limbic and paralimbic regions of recently abstinent methamphetamine abusers that correlated with self-reports of depression and anxiety.[14] Importantly, quantitative electroencephalographic (EEG) abnormalities consistent with generalized encephalopathy, i.e., increased EEG power in the delta and theta bands, have been reported in methamphetamine dependent subjects with 4 days of abstinence providing another evidence to the notion that methamphetamine abuse may be associated with a range of cognitive and psychiatric abnormalities.[15] Indeed, a preliminary finding of reduced cognitive function (assessed by Stroop test) in methamphetamine-dependent

subjects is consistent with distractibility that they show clinically.[16] Another study in methamphetamine-dependent subjects that have been abstinent for 8 months showed persistent abnormalities in cerebral flow that was accompanied by reduced cognitive function as tested by California Computerized Assessment Package.[17] Overall, methamphetamine abuse is associated with persistent physiological changes in the brain that are accompanied by reduced cognitive function.

4.1.2   Pharmacokinetics
Pharmacokinetics of methamphetamine is similar to those of ephedrine: it has high bioavailability, a long duration of action, and a significant fraction of methamphetamine is excreted unchanged in the urine. Following intravenous administration, methamphetamine is eliminated with a $t_{1/2}$ of 12 ± 3.2 hours.

4.1.3   Metabolism
Methamphetamine is metabolized by N-demethylation to amphetamine[18] and by hydroxylation to 4-OH methamphetamine.[19] Both of these reactions are catalyzed by cytochrome P450 2D6 (CYP2D6). Approximately 38% of the administered dose is excreted in the urine unchanged.[20] Methamphetamine and amphetamine also inhibit CYP2D6 with an apparent ki of 25 μM and 26.5 μM, respectively. [21] This could shift metabolism during chronic administration towards urinary excretion of the parent compound.

4.1.4   Short-term Effects of Methamphetamine Use
Methamphetamine is a powerful psychostimulant and even in small doses can increase wakefulness, attention and physical activity and decrease fatigue and appetite.[6] Those who smoke or inject methamphetamine report a brief, intense sensation, or rush. Oral ingestion or snorting produces a long lasting high instead of a rush, which reportedly can continue for as long as half a day. Both the rush and the high are the result of dopamine release in cortico-mesolimbic areas of the brain that regulate feeling of pleasure. High doses can elevate body temperature to dangerous, sometimes lethal levels, as well as cause convulsions.

4.1.5   Long-term Effects of Methamphetamine Use
Methamphetamine is an addictive drug. Long-term chronic methamphetamine abusers exhibit symptoms that can include violent behavior, anxiety, confusion, and insomnia. They also can display a number of psychotic features, including paranoia, auditory hallucinations, mood disturbances and delusions (for example, the sensation of insects creeping on the skin, which is called "formication"). The paranoia can result in homicidal as well as suicidal thoughts. With chronic use, tolerance for methamphetamine can develop. In an effort to intensify the desired effects, users may take higher doses of the drug, take it more frequently, or change their method of drug intake. In some cases, abusers forego food and sleep indulging in a form of binging known as "run," injecting as much as a gram of the drug every 2 to 3 hours over several days until the user runs out of the drug or is too disorganized to continue. Chronic abuse can lead to psychotic behavior, characterized by intense paranoia, visual and auditory hallucinations, and out-of-control rages that can be coupled with extremely violent behavior. These clinical data are confirmed by brain imaging studies that show long-term damage in dopaminergic and serotonergic neurons with a concomitant increase in glial cells in subjects with a history of methamphetamine abuse long after they stopped using methamphetamine.[13]

Methamphetamine abuse has a typical pattern of withdrawal manifested by signs and symptoms opposite to those produced by the drug. Users become sleepy, have a ravenous appetite, are exhausted, and may suffer from mental depression. This syndrome may last for several days after the drug is withdrawn. Tolerance develops quickly, so that abusers may take huge doses compared with those used medically, e.g., as anorexants.

4.1.6 Medical Complications of Methamphetamine Abuse

Methamphetamine toxicity manifests itself at the level of nearly every organ system with the most dramatic changes being observed in the cardiovascular system and brain. Methamphetamine can cause a variety of cardiovascular problems. These include rapid and sometimes irregular heartbeat,[22] increased blood pressure,[7] and irreversible, stroke-producing damage to small blood vessels in the brain.[23] Hyperthermia and convulsions occur with methamphetamine overdoses, and if not treated immediately, can result in death. Chronic methamphetamine abuse can result in endocarditis, and among users who inject the drug, damaged blood vessels and skin abscesses. Methamphetamine abusers also can have episodes of violent behavior, paranoia, anxiety, confusion, and insomnia. Psychotic symptoms can sometimes persist for months or years after use has ceased. Heavy methamphetamine users show progressive social and occupational deterioration.

4.1.7 Methamphetamine as a Major Health Problem

Methamphetamine has become a major drug of abuse in the United States since the early 1990's. High rates of methamphetamine dependence are also registered in Great Britain,[24] Japan,[25] Australia,[26-28] and in many other countries.[26] In Great Britain, the methamphetamine problem is considered of greater public health consequence than cocaine, especially in relation to HIV. In Australia, amphetamines are the second most frequently used drugs, after cannabis.

Methamphetamine abuse, long reported as the dominant drug problem in the San Diego, CA, area, has become a substantial drug problem in other sections of the West and Southwest, as well (NIDA Research Report on Methamphetamine, 2002). There are indications that it is spreading to other areas of the country, including both rural and urban sections of the South and Midwest. Methamphetamine, traditionally associated with white, male, blue-collar workers, is being used by more diverse population groups that change over time and differ by geographic area.29 According to the 2000 National Household Survey on Drug Abuse, an estimated 8.8 million people (4.0 % of the population) have tried methamphetamine at some time in their lives. Data from the 2000 Drug Abuse Warning Network (DAWN), which collects information on drug-related episodes from hospital emergency departments in 21 metropolitan areas, reported that methamphetamine-related episodes increased from approximately 10,400 in 1999 to 13,500 in 2000, a 30% increase. NIDA's Community and Epidemiology Work Group reported in June 2001, that methamphetamine continues to be a problem in Hawaii and in major Western cities, such as San Francisco, Denver and Los Angeles. Methamphetamine production and availability are being reported in more diverse areas of the country, particularly rural areas prompting concern about more widespread use.

Violence associated with methamphetamine (users under the influence, users who commit violent acts to obtain methamphetamine, and/or distributor-trafficker violence) is also a

concern.[30] Moreover, a generation of new users is engaging in highly risky sexual activities under the influence of methamphetamine, which raises the possibilities for a new wave of HIV transmission.

The lack of effective treatment for methamphetamine users has far reaching health ramifications both in terms of the consequences from continued drug use and from the potential for increased HIV transmission. As a result, the development of effective treatments for methamphetamine dependence has become a pressing concern for the national and global drug abuse treatment community.

4.1.8   Search for Effective Treatments for Methamphetamine Dependence

Despite a decade of intensive research, an effective pharmacotherapy for stimulant dependence remains elusive with a noted lack of controlled clinical trials in pharmacotherapy for methamphetamine abuse in particular.[31,32] To date, the bulk of the research in the field is oriented toward treatment of cocaine dependence and many of the suggestions on pharmacotherapies for methamphetamine abuse are based upon clinicians' experiences with treating cocaine abuse. The idea of applicability of cocaine treatment strategies for pharmacotherapy of methamphetamine dependence is based on the similarity of their pharmacological actions, i.e., cross-behavioral sensitization and tolerance between these psychostimulants in animal studies.[33-35] The concept of building on knowledge from cocaine dependence studies and applying this knowledge to methamphetamine studies was endorsed by the Methamphetamine Addiction Treatment Think Tank consultants meeting convened at NIDA on January 12, 2000.

Traditionally, the attempts to develop new medications to treat addiction were focused on the brain's "all-purpose" dopaminergic mesocorticolimbic reward area. However, recent reports indicate that the reward function operates independently from craving for a drug. This has been confirmed in a study by Vorel *et al.*[36] that anatomically located the relapse circuitry in the brain (i.e., ventral subiculum of hippocampus) and showed that the main chemical implicated is not dopamine but glutamate. Inhibition of prefrontal glutaminergic neurons blocks cocaine-induced reinstatement of drug seeking behavior in rats.[37,38] Preclinical studies have also suggested that medications that foster GABAergic neurotransmission reduce the dopamine response to both cocaine administration and to conditioned cues of prior cocaine use.[39-41] Also, increases in GABAergic activity induced by gamma-vinyl-GABA, an irreversible GABA transaminase inhibitor, have an attenuating effect on reward system and block cocaine self-administration in rats.[42,43] Conceptually, medications that inhibit glutaminergic activity and promote GABAergic activity may have a therapeutic potential for the treatment of cocaine and methamphetamine abuse.

4.1.9   Pharmacogenomics of Study Interventions and Genetics of Methamphetamine Dependence

Pharmacogenomics has the potential to identify sources of inter-individual variability in drug response (both efficacy and toxicity) in order to help individualize therapy with the intent of maximizing effectiveness and minimizing risk. The FDA is encouraging drug developers to conduct pharmacogenomic testing during drug development as a step toward this goal. This study will explore a broad range of genetic variants and gene expression profiles during

treatment in an attempt to establish a relationship between genetic variants and gene expression profiles with clinical response. Conduct of these studies will be dependent upon favorable efficacy findings.

Pharmacogenetic methods have been used to identify genes associated with positive outcomes following bupropion treatment in smoking cessation studies. Thus far, variants in the dopamine D2 receptor (DRD2) and catechol-O-methyltransferase (COMT) genes have been reported to be associated with positive response to bupropion in these studies.[44-47] Dopaminergic genes have been targeted in pharmacogenetic studies of bupropion, as bupropion has been implicated in inhibition of dopamine reuptake as well as norepinephrine reuptake. In addition, the A1 allele of the DRD2 gene (either homozygous A1/A1 or heterozygous A1/A2 allelles) is associated with a reduced number of dopamine binding sites in the brain and with the increased likelihood of substance abuse and addictive behavior.[48-50]

In an open-label, randomized efficacy study of 451 Caucasian smokers, Swan et al.[47] investigated whether variants in the DRD2 receptor gene were associated with smoking cessation outcomes following treatment with a combination of bupropion SR and behavioral counseling. Adherence to treatment and point-prevalent smoking status were assessed at 3 and 12 months, respectively, following a target quit date. Compared to women who carry both A2 alleles, women with at least one A1 allele were more likely to report having stopped taking bupropion due to medication side effects (odds ratio (OR)=1.91, 95% confidence interval (CI)=1.01-3.60; $P<0.04$) and at 12 months were somewhat more likely to report smoking (OR=0.76, 95% CI=0.56-1.03; $P<0.076$). Significant associations or trends were not observed in men. They concluded that in women, individual variability in responsiveness to bupropion-based treatment may be partially due to differences in genetic variants influencing dopamine receptor function.[47]

In a smaller randomized, placebo-controlled study of 30 smokers treated with bupropion, David et al.[45] reported that subjects treated with bupropion who reported significant attenuation of craving, irritability, and anxiety had the DRD2-A2/A2 genotype. In yet a third much larger (N=368), randomized controlled trial of bupropion compared to placebo control for smoking cessation, Lerman et al.[46] reported that at the end of the treatment phase, there was a statistically significant (p=0.01) interaction between the DRD2 -141C Insertion (Ins)/Deletion (Del)genotype and treatment indicated a more favorable response to bupropion among smokers homozygous for the Ins C allele compared to those carrying a Del C allele.

COMT is a key enzyme involved in the metabolic inactivation of dopamine. The G to A transition in codon 158/108 of COMT converts a valine high activity allele to methionine low-activity allele, resulting in a 3- to 4-fold reduction in COMT activity.[51] The methionine allele is associated with decreased brain enzyme levels[52] and improved cognitive performance in prefrontal cortex tasks, such as memory tests.[53] Berrettini et al.[44] examined 3 COMT SNPs on the association between bupropion treatment and smoking cessation in a placebo-controlled, randomized, double blind trial of 541 smokers. Smokers of European-American origin with the GG COMT haplotype for both rs737865 and rs165599 did not benefit from bupropion treatment; however, those with an A allele at rs165599 had a significantly probability of a benefit.[44]

Other genes that may be associated with bupropion efficacy and safety include those genes that code for cytochrome P450 (CYP) enzymes responsible for bupropion metabolism. Bupropion is primarily metabolized in human liver by CYP 2B6, an isoform that shows high interindividual variability in expression and catalysis which has been hypothesized to account for the variability in treatment response and toxicity associated with bupropion.[54,55] Rapid clearance of bupropion via hydroxylation could account for treatment failure, likewise, slow clearance could account for toxicity. Recently, the 6B haplotype of CYP 2B6 has been shown to be a significant predictor of bupropion hydroxylation in human liver microsomal preparations, thus suggesting that individuals with this haplotype may be poorer responders to treatment.[54]

The rationale for screening for genetic variants stems from family and twin studies suggesting that substance abuse and addiction are complex traits that are influenced by genetics, the environment and their interaction. Support for this concept is provided from a large study of twins, where the concordance rates of DSM-III-R-defined drug abuse or dependence for monozygotic twins were almost double the rates for dizygotic twins.[56] There are a number of plausible candidate genes for methamphetamine dependence — that is, genes likely to affect an individual's vulnerability to methamphetamine dependence. Among the candidate genes operative in addiction disorders are serotonin, norepinephrine, human γ-aminobutyric acid (GABA), glutamate, and opioid receptors, all of which modify dopamine metabolism and dopamine neurons. It has been proposed that defects in various combinations of the genes for these neurotransmitters result in a reward deficiency syndrome and that such individuals are at risk for abuse of the "unnatural rewards."[57]

Already a number of genes have been identified that appear to be associated with a predisposition for methamphetamine dependence and psychosis including COMT,[58] the dopamine DRD4 receptor,[58] the μ-opioid receptor gene,[59] OPRM1, gamma-aminobutyric acid receptor gamma 2 subunit gene (GABRG2),[60] the prodynorphin gene,[61] and the β-arrestin 2 gene.[62] Thus, in this study, we plan to investigate the potential association of genetic variants of a number of genes with methamphetamine dependence. Given that only limited number of candidate genes have been investigated in their potential association with methamphetamine dependence, we plan to use Affymetric 500K Array set to identify susceptibility genes for methamphetamine dependence, with the special emphasis on those genes involved in the dopaminergic reward system, such as the genes encoding tyrosine hydroxylase, dopamine transporters, dopamine receptors, and monoamine oxidase A and B. Additional genes of interest include those encoding serotonin transporters, opioid receptors, and cannabinoid receptors.

Several recent findings about the activity and genetics of BDNF are pertinent to our present study. First, BDNF has been shown to promote cocaine seeking and to increase vulnerability to relapse in rats.[63] BDNF injections into the accumbens shell produced stronger reinstatement of cocaine self-administration after extinction, using various stimuli of cocaine priming, cocaine cues, or stress. Second, in the Val66Met polymorphism of the human BDNF-gene, the 66Val allele is associated with higher BDNF secretion in response to neuronal stimulation compared to the 66Met allele.[64] This BDNF 66Val homozygote was found to have a higher frequency in people with drug addiction than in normal controls.[65] Also, plasma BDNF concentrations in methamphetamine users were significantly higher than in controls.[66] Finally, chronic bupropion treatment significantly decreased BDNF expression in the dentate gyrus of the hippocampus in

rats.[67] Taken together, these findings lead us to hypothesize that methamphetamine users who decrease their drug use across the study drug administration period (regardless of receiving bupropion) will have a decrease in plasma BDNF, while those who do not reduce methamphetamine use regardless of whether they receive bupropion or placebo will have no change. In addition, methamphetamine users who have the BDNF 66Val homozygote will be more likely to benefit from bupropion and decrease drug use than those who do not.

Additionally, we plan to study gene expression profiles of subjects in response to bupropion over different time points. Methods to define the patterns of gene expression have been applied to a wide range of biological systems including substances of abuse (for a review, see Li *et al*.[68]). One approach to understanding physiological mechanisms is to identify gene expression patterns associated with varying physiological states. For example, investigators have been interested in examining differentially expressed genes in different cell types, in cells during different stages of differentiation and under various growth conditions, and during drug treatment versus controls. Various methods to compare patterns of gene expression have been reported, including differential hybridization screening, [69] differential display, [70] series analysis of gene expression, [71,72] and cDNA microarray.[73,74]

The techniques of differential display and the generation of expressed sequence tags (ESTs) were first used for the identification of genes exhibiting marked differential expression across tissues, developmental stages, or normal versus pathological conditions. The analysis of gene expression patterns derived from normal and pathological situations is a valuable tool in the discovery of therapeutic targets and diagnostic markers. The recognition of coordinated expression profiles between characterized or anonymous genes also enables inferences about biological pathways and gene functions. Microarray technology, which facilitates the measurement of the relative gene expression levels through a massively parallel approach, has begun to revolutionize biomedical research. The technology behind microarray was developed over the last several years once it became apparent that new, more powerful analytical approaches were needed to utilize the flood of genomic data and resources being acquired through the various genomic projects. At the moment, the measurement of gene expression using microarray appears to be the sole approach to gene characterization capable of matching the speed of sequencing and the scale required for functional genomics.

Array technologies have made it straightforward to simultaneously monitor the expression patterns of thousands of genes during different experimental conditions. Protocols have become more refined, so good quality of microarray data can now be obtained. The greatest challenge that researchers face is to make sense of such massive data sets. Many mathematical techniques have been developed for identifying the underlying patterns in a complex data set. Currently, the most popular way to identify interesting genes and their function is to perform cluster analysis on the relative expression pattern changes obtained from a typical microarray experiment. However, it is not clear which clustering technique(s) is likely to be most useful for interpreting gene expression data. Hierarchical clustering is the most commonly used method, [75-77] in which data points are forced into a strict hierarchy of nested subsets with branch lengths reflecting the degree of similarity in expression. Several other clustering techniques also have been applied to the analysis of gene expression patterns, which include k-means clustering,[78] self-organizing maps,[79] and quality clustering.[80]

There is no doubt that cluster analysis contributes significantly to our understanding of the underlying biological phenomena in differential gene expression. Primarily, cluster analysis has been used for data reduction and visualization and can be used to generalize or predict the categorization of new samples (for a review, see Slonim[81]). Further, clues to unknown gene function may be inferred from clusters of genes similarly expressed across many samples.[76] Clustering samples over the expression levels of multiple genes also has been proposed as a way of defining new disease subclasses.[82,83] However, most of these methods have some restrictions, one of which is their inability to determine accurately the number of clusters. The difficulty may be related to the fact that, in many methods, there is no clear definition of what constitutes a cluster in the first place. Furthermore, the clustering results from these methods may not be stable.[84,85] An important clustering technique that improves and provides alternative solutions to these issues is the model-based clustering approach, [86] which has been applied to cluster gene-expression patterns.[87,88] Another concern in microarray data clustering is related to the assumptions made when interpreting the results obtained using these methods. The fundamental premise of the clustering approach is that genes having similar expression profiles across a set of experimental conditions also may share similar functions. As we know, this assumption may not be true in all cases. Genes, the products of which may have the same function, do not necessarily share similar transcriptional patterns. Conversely, genes having different functions can have a similar expression profile simply by chance. To overcome this limitation, a novel approach, called shortest path analysis, recently has been proposed to group genes involved in the same biological process, even without showing significant expression similarity.[89]

In addition to the expression pattern discovery, identification of genes that are differentially expressed under varying experimental conditions is equally important to molecular biologists that are using the microarray technique to address the biological questions of their interest. In earlier microarray studies, a fixed fold-change cut-off (generally two-fold) was used to identify the genes exhibiting the most significant variation. This set value is arbitrary and sometimes unreliable since it does not take individual variability into account. Recently, more sophisticated statistical methods have been proposed to overcome this shortcoming, including analysis of variance,[90-92] maximum likelihood analysis,[93] a regression modeling approach,[94] an empirical Bayes method,[19] and a Significance Analysis of Microarray method.[95]

Since its development, the microarray technique has revolutionized almost all fields of biomedical research by enabling high-throughput gene expression profiling. Using cDNA or oligonucleotide microarrays, thousands of genes from various organisms have been examined with respect to differentiation/development, disease diagnosis, and drug discovery. Nevertheless, research on drug addiction using the microarray approach has been rather limited. Therefore, the current study provides a unique opportunity for us to determine which sets of genes are significantly modulated by bupropion and how their expression levels are changed over different time points during its administration. Such information may shed light on the understanding of molecular mechanisms underlying the pharmacological effects of bupropion in individuals with methamphetamine dependence.

4.2 Bupropion

4.2.1 Rationale for Studying Bupropion

Bupropion is an attractive candidate medication for the treatment of methamphetamine dependence for several reasons:

1. Bupropion is an antidepressant with stimulant properties[96] that is effective for the treatment of nicotine dependence.[97]
2. In a clinical trial of bupropion for cocaine dependence[98] an exploratory analysis suggested that patients with greater levels of depression may have benefited the most. The association with improvement in patients with depression is promising for methamphetamine dependence as clinically, acute abstinence from methamphetamine in chronic users is associated with depression and impaired concentration.[99]
3. Bupropion's pharmacologic activities are thought to operate through a dopaminergic mechanism which when combined with its ability to alleviate the dysphoria seen in early abstinence may reduce craving, thus helping to prevent relapse.
4. Bupropion was found to be safe in clinical laboratory studies assessing its effects when administered concurrently with intravenous methamphetamine.[100,101]
5. A Phase 2 efficacy study showed that bupropion significantly decreased methamphetamine use in methamphetamine dependent subjects who were using methamphetamine 18 days or less in the 30-day period prior to the start of screening (described below).

Bupropion is an antidepressant of aminoketone class that is chemically unrelated to tricyclic, tetracyclic, selective serotonin reuptake inhibitors (SSRIs), or other known antidepressant agents. The mechanism of its antidepressant action may be related to its mild dopaminergic activity. Bupropion is considered to be a dopaminergic antidepressant based on its ability to inhibit the uptake of dopamine more selectively than it inhibits uptake of norepinephrine or serotonin. Bupropion has a favorable side effect profile: it causes fewer anticholinergic, cardiovascular, sedative or adverse sexual effects than tricyclics and does not cause weight gain.[102]

Bupropion is a relatively weak inhibitor of the neuronal reuptake of dopamine and thus has a low abuse liability.[103] Its potency to block dopamine reuptake in animals manifests at doses higher than those necessary for its antidepressant effect. It is possible however that the mild dopaminergic activity that bupropion does possess is sufficient to exert anti-craving effect and to treat the signs of withdrawal.

Bupropion has been proved to be effective for treatment of nicotine dependence[104,105] and is FDA-approved and marketed as Zyban, a non-nicotine aid to smoking cessation. Bupropion has been investigated for treatment of cocaine abuse. In a pilot study, Margolin *et al.*[106] found that bupropion 300 mg/day, administered to five cocaine-dependent methadone-maintenance patients substantially reduced cocaine use in four of the five, was well tolerated and reduced self-reported craving for cocaine. A multicenter placebo-controlled double-blind clinical trial of bupropion for cocaine dependence in methadone-maintenance patients indicates efficacy of bupropion for the subgroup of patients with depression at study entry.[98]

The results of animal studies designed to test the ability of bupropion to block effects of methamphetamine support clinical use for the treatment of methamphetamine dependence.[107]

Thus, bupropion provides complete protection against methamphetamine-induced decrease in dopamine uptake in striatum in *in vitro* model of methamphetamine-induced dopamine nerve terminal toxicity. It is logical to postulate that combination of bupropion's dopaminergic activity and antidepressant properties may guarantee its efficacy in clinical trials for treatment and relief of the signs and symptoms of methamphetamine withdrawal and for prevention of the relapse.

4.2.2 Molecular Mechanism for Bupropion's Potential Efficacy for Methamphetamine Dependence

Bupropion may be effective for methamphetamine dependence due to its ability to counteract methamphetamine-induced alterations in molecules regulating synaptic dopamine disposition. Recent studies have shown that psychostimulants produce their effects on dopaminergic neurotransmission via disruption of key molecules responsible for regulating the disposition of dopamine within the synapse and neuronal cytoplasm. Normally, dopamine released from synaptic vesicles in response to an action potential is cleared from the synapse by re-uptake into the presynaptic neuron via the dopamine transporter DAT and/or by metabolism by the enzyme COMT. After re-uptake into the cytoplasm, dopamine is sequestered into synaptic vesicles by transport via vesicular monoamine transporter-2 (VMAT-2), in order to prevent the generation of toxic reactive oxygenation species (ROS) that are produced by accumulation of dopamine within the cytoplasm. Further protection against ROS-induced toxicity is provided by the glutathione S-transferase family of enzymes, which catalyze conjugation of reduced glutathione with ROS that are generated.

Figure 1: Effect of Releasers and Uptake Blockers on dopamine (DA) disposition

Releasers (MA): VMAT-2 disruption, cytosolic DA accumulation, reactive oxygen species (ROS) production

Uptake Blockers (Bupropion): DAT blockade, sequestration of DA in vesicles, reduced ROS production

Psychostimulants cause an acute increase in synaptic dopamine concentrations and can be divided into two groups, which Hanson and colleagues have termed "releasers" and "uptake blockers," based on their mechanism of action.[108] Releasers (**Figure 1**), such as methamphetamine, increase synaptic dopamine levels by producing increased release of dopamine from presynaptic neurons. Methamphetamine is highly lipophilic and thereby diffuses into synaptic vesicles where it disrupts the vesicular proton gradient necessary for functioning of VMAT-2, resulting in impaired sequestering of cytosolic dopamine into vesicles.[109,110] As a result, dopamine accumulates in the cytoplasm leading to increased release of dopamine into the synapse by reverse transport via DAT.[110,111] In addition to disrupting VMAT-2 function, MA also causes trafficking of VMAT-2 containing vesicles out of the neuronal cytoplasm, thereby reducing the pool of synaptic vesicles available to sequester dopamine and further increasing cytoplasmic dopamine accumulation.[112] While the mechanism by which releasers cause this vesicular trafficking is not clear, activation of D2 dopamine receptors (DRD2) is required,[113] suggesting that methamphetamine-induced increases in synaptic dopamine may activate presynaptic DRD2 receptors which then trigger vesicular trafficking.

In addition to these molecular changes, chronic methamphetamine use also produces significant damage to dopaminergic neurons and deficits in dopaminergic function. Preclinical studies have shown that high-dose methamphetamine exposure produces neurotoxic changes among dopaminergic cells in the striatum as well as deficits in striatal tyrosine hydroxylase activity, dopamine concentrations, and DAT levels. [114-118] Clinical imaging studies have also shown significant deficits in dopaminergic function among chronic methamphetamine users, including reductions in DAT density [11] and dopamine receptor occupancy, [4,119] which are thought to contribute to the clinical symptoms that accompany methamphetamine dependence and withdrawal. This methamphetamine-induced neurotoxicity and the resulting deficits in dopaminergic function result in part from harmful intracellular ROS that are produced when dopamine accumulates in the cytoplasm. [120,121] As a result, medications that counteract the methamphetamine-induced molecular/cellular changes that produce dopamine accumulation, including methamphetamine-induced disruptions in VMAT-2 function, have potential as treatments for methamphetamine dependence due to their ability to minimize damage due to disruptions in dopaminergic function produced by chronic methamphetamine use.

Reuptake blockers (**Figure 1**), such as the antidepressant bupropion, bind to DAT, thereby blocking transport of dopamine from the synapse into the cytoplasm, and increasing synaptic dopamine levels. [122,123] In addition, reuptake blockers also increase VMAT-2 function and trigger trafficking of VMAT-2 containing vesicles into the cytoplasm of presynaptic neurons again via an unknown DRD2 mediated mechanism, thereby increasing vesicular dopamine uptake. [124,125] As a result, cytoplasmic accumulation of dopamine and subsequent ROS formation does not occur with reuptake blockers, and furthermore as a result of their effect on VMAT-2, reuptake blockers may be effective in reversing the disruptions in VMAT-2 functioning and subsequent neurotoxicity caused by releasers including methamphetamine. In fact, preclinical studies have shown that post-treatment with the reuptake blockers methylphenidate and bupropion after treatment with methamphetamine did reverse the methamphetamine-induced reductions in VMAT-2 activity, but ***bupropion did not*** prevent the long-term deficits in dopaminergic function produced by repeated high-dose methamphetamine administration. [125,126] While the reasons for this have not been established experimentally, frequent high-dose methamphetamine use may simply overcome bupropion's ability to counteract methamphetamine-induced alterations in dopamine disposition.

In summary, methamphetamine has profound effects on molecules that regulate synaptic dopamine disposition and acute methamphetamine administration results in release of dopamine which is thought to contribute to the subjective effects of methamphetamine. Frequent high-dose methamphetamine use results in accumulation of toxic ROS that contribute to the deficits in dopaminergic function that are seen clinically in chronic methamphetamine users. Dopamine re-uptake inhibitors, such as bupropion, may be effective treatments for methamphetamine dependence due to their ability increase synaptic dopamine levels, thereby ameliorating methamphetamine-induced deficits in dopaminergic function, and to counteract methamphetamine-induced alterations in dopamine disposition and subsequent ROS formation via enhanced vesicular dopamine uptake. Yet the failure of bupropion to prevent long-term dopaminergic deficits after repeated high-dose methamphetamine in preclinical studies suggests that bupropion's clinical utility for methamphetamine dependence may be limited to low frequency methamphetamine users.

4.2.3 Bupropion Pharmacology

Pharmacokinetics. Tablets of bupropion hydrochloride come in immediate- and SR formulations. The bupropion SR, 150 mg, twice daily formulation will be used for this study. Bupropion SR has been shown to be bioequivalent to 100 mg three times daily of the immediate release formulation with regards to rate and extent of absorption and parent drug and metabolites in clinical trials for depression. Better compliance is expected with twice daily dosing as opposed to thrice daily dosing making bupropion SR an obvious choice for study.

The half-life of bupropion is approximately 21 hours after chronic dosing. Peak plasma concentrations of bupropion are achieved within 3 hours following oral administration. The mean peak concentration (C_{max}) values were 91 and 143 ng/mL from 2 single-dose (150-mg) studies. At steady state, the mean C_{max} following a 150-mg dose every 12 hours is 136 ng/mL. In a single-dose study, food increased the C_{max} of bupropion by 11% and the extent of absorption as defined by area under the plasma concentration-time curve (AUC) by 17%. The mean time to peak concentration (T_{max}) was prolonged by 1 hour. This effect was of no clinical significance. Steady state plasma concentrations of bupropion are reached within 8 days. Four basic metabolites have been identified. These metabolites are pharmacologically active, but their potency and toxicity relative to bupropion have not been totally characterized. They may be of clinical importance because the plasma concentration of metabolites is higher than those of bupropion.

Bupropion is a racemic mixture. The pharmacologic activity and pharmacokinetics of the individual enantiomers have not been studied. Bupropion follows biphasic pharmacokinetics best described by a 2-compartment model. The terminal phase has a mean half-life ($\pm\%$ CV) of about 21 hours ($\pm20\%$), while the distribution phase has a mean 57 half-life of 3 to 4 hours.

In vitro tests show that bupropion is 84% bound to human plasma proteins at concentrations up to 200 µg/mL. The extent of protein binding of the hydroxybupropion metabolite is similar to that for bupropion, whereas the extent of protein binding of the threohydrobupropion metabolite is about half that seen with bupropion. The volume of distribution (V_{ss}/F) estimated from a single 150-mg dose given to 17 subjects is 1,950 L (20% CV).

Metabolism. Bupropion is extensively metabolized in humans. Three metabolites have been shown to be active: hydroxybupropion, which is formed via hydroxylation of the *tert*-butyl group of bupropion, and the amino-alcohol isomers threohydrobupropion and erythrohydrobupropion, which are formed via reduction of the carbonyl group. *In vitro* findings suggest that CYP2B6 is the principal isoenzyme involved in the formation of hydroxybupropion, while cytochrome P450 isoenzymes are not involved in the formation of threohydrobupropion. Oxidation of the bupropion side chain results in the formation of a glycine conjugate of meta-chlorobenzoic acid, which is then excreted as the major urinary metabolite. The potency and toxicity of the metabolites relative to bupropion have not been fully characterized. However, it has been demonstrated in an antidepressant screening test in mice that hydroxybupropion is one half as potent as bupropion, while threohydrobupropion and erythrohydrobupropion are 5-fold less potent than bupropion. This may be of clinical importance because the plasma concentrations of the metabolites are as high or higher than those of bupropion.

Because bupropion is extensively metabolized, there is the potential for drug-drug interactions, particularly with those agents that are metabolized by the CYP2B6 isoenzyme. Although bupropion is not metabolized by CYP2D6, there is the potential for drug-drug interactions when bupropion is co-administered with drugs metabolized by this isoenzyme. Following a single dose in humans, peak plasma concentrations of hydroxybupropion occur approximately 6 hours after administration of bupropion SR. Peak plasma concentrations of hydroxybupropion are approximately 10 times the peak level of the parent drug at steady state. The elimination half-life of hydroxybupropion is approximately 20 (±5) hours, and its AUC at steady state is about 17 times that of bupropion. The times to peak concentrations for the erythrohydrobupropion and threohydrobupropion metabolites are similar to that of the hydroxybupropion metabolite; however, their elimination half-lives are longer, 33 (±10) and 37 (±13) hours, respectively, and steady-state AUCs are 1.5 and 7 times that of bupropion, respectively. Bupropion and its metabolites exhibit linear kinetics following chronic administration of 300 to 450 mg/day.

4.2.4   Previous Human Experience with Bupropion in Methamphetamine Dependence

Phase 1 and Phase 2 clinical trials assessing the safety and efficacy of bupropion for methamphetamine dependence have been conducted with positive outcomes. In a Phase 1 clinical laboratory study, 26 subjects with a diagnosis of methamphetamine dependence or abuse by DSM-IV criteria were randomized in a double-blind in-patient study to receive either twice daily bupropion SR (150 mg BID) or placebo control and infusions of saline or 15 mg and 30 mg of methamphetamine. Of the 26 subjects who were enrolled, 20 completed the entire study with 10 subjects in each of the bupropion and placebo control groups. Bupropion treatment was associated with reduced ratings of "any drug effect" (p=0.02), and "high" (p=0.02) that were assessed with visual analog scales following methamphetamine administration. Bupropion also significantly reduced cue-induced craving [General Craving Scale total score (p=0.002); Behavioral Intention subscale (p=0.001)].[100] Bupropion treatment was well tolerated, with the bupropion- and placebo-treated groups reporting similar rates of AEs. Methamphetamine administration was associated with the expected stimulant cardiovascular effects, and these were not accentuated by bupropion treatment. Instead, there was a trend for bupropion to reduce methamphetamine-associated increases in blood pressure and a statistically significant reduction in methamphetamine-associated increases in heart rate. Pharmacokinetic analysis revealed that bupropion treatment reduced the plasma clearance of methamphetamine and also reduced the appearance of amphetamine in the plasma. Methamphetamine administration did not alter the peak and trough plasma concentrations of bupropion or its metabolites.[101]

NIDA conducted a Phase 2 study evaluating bupropion for methamphetamine dependence (Protocol Number CTO-0008). This study was of similar design to the present study, except that subjects in the first study were selected from a group of methamphetamine users that was not restricted to low use prior to the start of the study (i.e., low use being defined in the current study population as ≤ 18 days of methamphetamine use in the 30 day period before the start of screening). The CTO-0008 study was a double-blind, placebo-controlled, randomized, two arm parallel-group study comparing 150 mg of bupropion to placebo administered twice daily to methamphetamine dependent outpatients. One hundred one (151) subjects were in the ITT population, of which 121 subjects were considered evaluable. The major conclusions regarding the efficacy of bupropion in this study were as follows:

1. Analysis of the primary outcome measure (weekly proportion of subjects with all urine specimens free of methamphetamine) showed a trend toward statistical significance favoring bupropion over placebo for the entire population (GEE, $p=0.09$).
2. Bupropion showed a statistically significant effect for weekly methamphetamine-free week in subjects who used methamphetamine 18-days or less in the 30 days prior to the start of screening (GEE, $p<0.04$).
3. More subjects in the bupropion group than the placebo group reduced their proportion of methamphetamine use days to 50% or less of their baseline rate based on self report only (Chi-square test, $p=0.02$).
4. Bupropion showed a statistically significant effect for weekly methamphetamine-free week in males (GEE, $p=0.04$).
5. Bupropion showed a trend toward significance favoring bupropion for methamphetamine-free week in non-depressed subjects (GEE, $p=0.08$).
6. Bupropion showed a statistically significant effect for weekly methamphetamine-free week in subjects without ADD (GEE, $p=0.02$).

In this study, subjects were randomized to receive thrice weekly CBT and either 150 mg of twice daily bupropion SR or placebo control with one of the randomization variables the frequency of methamphetamine use, by self-report, in the 30 days prior to the start of screening based on two rates of use: 1) low rate users had 18 of less days of methamphetamine use; 2) high rate users had 19 or more days of methamphetamine use. Forty seven percent (47%) of the ITT population were low rate users and 53% were high rate users. A GEE analysis was performed on the primary outcome measure for each of these two study subpopulations as well as for the entire ITT population. This subset analysis showed that the group of low rate users administered bupropion had a statistically significant increase (GEE, $p<0.04$) in the slope of weekly proportion of subjects with methamphetamine negative urines compared to the group to the low rate users administered placebo. The high rate of methamphetamine users appears to have diluted out the bupropion effect when examining the entire study population.

Figure 2 illustrates the frequency of abstinence over the last two weeks in non-daily methamphetamine users in the bupropion study where 15 out of 63 subjects (23.8%) achieved abstinence as compared to 3 out of 53 (5.7%) in the placebo group. This finding was shown to be statistically significant ($p=0.01$, Chi square test). Although there was a fairly high drop-out rate over the course of the study (approximately 50% of the subjects completed the study), it is important to note that there were no significant differences in the rate of drop-outs (conversely study retention) between the two groups
(**Figure 3**).

Bupropion was well tolerated in this study. The only significant treatment emergent AE was nausea. Subjects administered bupropion had a significant increase (Chi-square, $p=0.019$) in the incidence of nausea over the intervention period. One subject (a 31 year old female) in the bupropion group temporarily discontinued taking bupropion during the study due to an AE. Twenty six days after taking bupropion, this subject reported having nausea, vomiting, and diarrhea that persisted for 3 to 4 days. She took Pepto Bismol and Motrin during this same period and stopped taking bupropion for 4 days. The investigator determined that this AE was definitely

not related to the study drug and study interventions were resumed after resolution of gastrointestinal symptoms. The subject completed the study.

Figure 2. Abstinence for the last two weeks Non-daily user in Bupropion study

Non-Daily users			
Group	**Abstinence Last 2 Weeks**		
Frequency (Percent)	**fail**	**success**	**Total**
Bupropion	48 (76.2%)	15 (23.8%)	63
Placebo	50 (94.3%)	3 (5.7%)	53
Total	98	18	116

P=0.01 (Chi-Square test)

Figure 3. Rate of Retention in Phase 2 Study of Bupropion for Methamphetamine Dependence
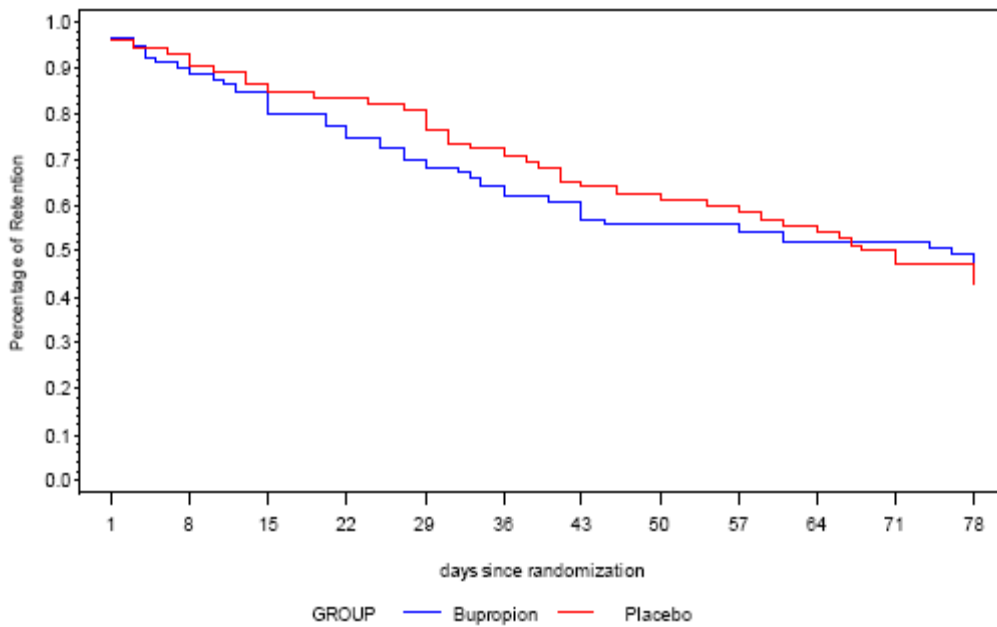
Three serious adverse events (SAEs) were reported in this study, all of which were psychiatric disorders. One subject (a 36 year old male) with an SAE who received bupropion was hospitalized on April 26, 2005 and placed under an involuntary 72-hour inpatient observation period due to psychiatric instability. This subject was diagnosed with Bipolar Disorder. This SAE resolved without sequelae and was determined by the investigator definitely not related to

bupropion. The other two subjects with SAEs received placebo. One, a 29 year old male in the placebo group, was admitted to a medical center for five days with the diagnosis of suicidal ideation after excessive daily intravenous methamphetamine use. The other, a 35 year old male in the placebo group, was committed to a medical center after study termination, for hallucinations.

4.2.5 Safety of Bupropion

Contraindicated Conditions. Bupropion (ZYBAN Package Insert March 2007) is contraindicated in subjects:

1. With a seizure disorder.
2. Who are concurrently using a bupropion containing medication because the incidence of seizure is dose dependent.
3. Who have a current or prior diagnosis of bulimia or anorexia nervosa because of a higher incidence of seizures noted in patients treated for bulimia with the immediate-release formulation of bupropion.
4. Who are abruptly discontinuing alcohol or sedatives (including benzodiazepines).
5. Who are concurrently taking a monoamine oxidase (MAO) inhibitor (at least 14 days should elapse between discontinuation of an MAO inhibitor and initiation of bupropion administration).
6. Who have had an allergic response to bupropion.
7. Who have major depressive disorder (patients may experience worsening of their depression and/or the emergence of suicidal ideation and behavior (suicidality) or unusual changes in behavior, whether or not they are taking antidepressant medications, and this risk may persist until significant remission occurs). There has been a long-standing concern that antidepressants may have a role in inducing worsening of depression and the emergence of suicidality in certain patients.

AEs Associated With the Discontinuation of Treatment. AEs leading to discontinuation of treatment with bupropion SR included nervous system disturbances 655 (3.4%), primarily tremors, and skin disorders (2.4%), primarily rashes.

Incidence of Commonly Observed Adverse Events. The most commonly observed AEs (those that consistently occurred at a rate of 5 percentage points greater than that for placebo across clinical studies) consistently associated with the use of bupropion SR were dry mouth and insomnia.

Risk of Seizure. Predisposing factors that may increase the risk of seizure with bupropion use include history of head trauma or prior seizure, central nervous system (CNS) tumor, the presence of severe hepatic cirrhosis, and concomitant medications that lower seizure threshold. Circumstances associated with an increased seizure risk include, among others, excessive use of alcohol or sedatives (including benzodiazepines); addiction to opiates, cocaine, or stimulants; use of over-the-counter stimulants and anorectics; and diabetes treated with oral hypoglycemics or insulin. Concomitant medications such as antipsychotics, antidepressants, theophylline, and systemic steroids are known to lower seizure threshold. The risk of seizure may be minimized if

the total daily dose of bupropion SR dose not exceed 300 mg, which is the dose planned in this study.

In addition, bupropion SR should be administered with extreme caution to subjects with a history of seizure, cranial trauma, or other predisposition(s) toward seizure, or patients treated with other agents (e.g., antipsychotics, antidepressants, theophylline, systemic steroids, etc.) that lower seizure threshold. These subjects will be excluded from participation in the study; moreover, these medications are prohibited during the intervention phase of the study.

Allergic Reactions. Anaphylactoid/anaphylactic reactions characterized by symptoms such as pruritus, urticaria, angioedema, and dyspnea requiring medical treatment have been reported at a rate of about 1 to 3 per thousand in clinical trials of bupropion SR. In addition, there have been rare spontaneous postmarketing reports of erythema multiforme, Stevens-Johnson syndrome, and anaphylactic shock associated with bupropion. Subjects will be advised to stop taking investigational products and consult a doctor if experiencing allergic or anaphylactoid/ anaphylactic reactions (e.g., skin rash, pruritus, hives, chest pain, edema, and shortness of breath) during treatment. Arthralgia, myalgia, and fever with rash and other symptoms suggestive of delayed hypersensitivity have been reported in association with bupropion. These symptoms may resemble serum sickness.

Insomnia. In the dose-response smoking cessation trial, 29% of patients treated with 150 mg/day of bupropion SR and 35% of patients treated with 300 mg/day of bupropion SR experienced insomnia, compared to 21% of placebo-treated patients. Symptoms were sufficiently severe to require discontinuation of treatment in 0.6% of patients treated with bupropion SR and none of the patients treated with placebo.

Neuropsychiatric Side Effects. In clinical trials with bupropion SR conducted in nondepressed smokers, the incidence of neuropsychiatric side effects was generally comparable to placebo. Depressed patients treated with bupropion in depression trials have been reported to show a variety of neuropsychiatric signs and symptoms including delusions, hallucinations, psychosis, concentration disturbance, paranoia, and confusion. In some cases, these symptoms abated upon dose reduction and/or withdrawal of treatment.

Antidepressants can precipitate manic episodes in bipolar disorder patients during the depressed phase of their illness and may activate latent psychosis in other susceptible individuals. The SR formulation of bupropion is expected to pose similar risks.

Cardiovascular Conditions. In clinical practice, hypertension, in some cases severe, requiring acute treatment, has been reported in patients receiving bupropion SR alone and in combination with nicotine replacement therapy. These events have been observed in both patients with and without evidence of preexisting hypertension. Data from a comparative study of ZYBAN, nicotine transdermal system (NTS), the combination of bupropion SR plus NTS, and placebo as an aid to smoking cessation suggest a higher incidence of treatment-emergent hypertension in patients treated with the combination of bupropion SR and NTS. In this study, 6.1% of patients treated with the combination of bupropion SR and NTS had treatment-emergent hypertension compared to 2.5%, 1.6%, and 3.1% of patients treated with bupropion SR, NTS, and placebo,

respectively. The majority of these patients had evidence of preexisting hypertension. Three patients (1.2%) treated with the combination of bupropion SR and NTS and 1 patient (0.4%) treated with NTS had study medication discontinued due to hypertension compared to none of the patients treated with bupropion SR or placebo.

Subjects will be asked to inform a study physician, if they decide to use any smoking cessation products such as nicotine gum, nicotine patch, nicotine nasal spray or nicotine inhaler while on study. If any agents are used, blood pressure should be monitored closely as bupropion may increase blood pressure when used with these smoking cessation agents.

Drug Interactions. *In vitro* studies indicate that bupropion is primarily metabolized to hydroxybupropion by the CYP 2B6 isoenzyme. Therefore, the potential exists for a drug interaction between bupropion SR and drugs that are substrates or inhibitors of the CYP 2B6 isoenzyme (e.g., orphenadrine, thiotepa, and cyclophosphamide). In addition, *in vitro* studies suggest that paroxetine, sertraline, norfluoxetine, and fluvoxamine as well as nelfinavir, ritonavir, and efavirenz inhibit the hydroxylation of bupropion. No clinical studies have been

performed to evaluate this finding. The threohydrobupropion metabolite of bupropion does not appear to be produced by the CYP isoenzymes.

Because bupropion is extensively metabolized, the coadministration of other drugs may affect its clinical activity. In particular, certain drugs may induce the metabolism of bupropion (e.g., carbamazepine, phenobarbital, phenytoin), while other drugs may inhibit the metabolism of bupropion (e.g., cimetidine).

Reproductive Toxicology. Bupropion is a pregnancy Category C drug.

4.3   Psychosocial Interventions for Methamphetamine Dependence

Combinations of pharmacologic interventions with psychosocial counseling has become a standard approach in drug development trials due to the additive or synergistic efficacy of combined modalities. Methamphetamine users tend to have poor treatment retention, with only 42% completing the course of treatment and nearly 60% relapsed in the year following treatment.[127] Psychosocial counseling improves treatment retention and thus may be a key element in pharmacologic intervention studies. This is supported by analysis of the smoking cessation literature, in which it was found that adding behavioral therapy to nicotine replacement, doubled the quit rate evident with replacement therapy alone and vice versa. Adding nicotine replacement to behavioral therapy essentially doubled smoking cessation rates.[127] Similarly, the heroin, cocaine and alcohol addiction treatment literature shows good results from the combination of medication and behavioral interventions.[128-130] The combination of behavioral interventions with methadone treatment results in reduced opiate use and increased attendance/retention rates.[25,131-139] Overall, the existing evidence for combining medication and behavioral interventions indicates the efficacy of this approach in the treatment of methamphetamine dependence.[140]

4.4   Psychosocial Intervention

In contrast to opioid dependence treatment trials in which medication effects are large, methamphetamine dependence prevention trials require psychosocial interventions that are robust enough to make participants return to the clinic. Studies with inadequate psychosocial support may suffer from excessive early termination, which distorts conclusions regarding the efficacy of medication.[31] Thus, use of adequate CBT strategies is very important. CBT was developed during the 1980's and is based on a set of social learning theory techniques. These techniques refer to a wide range of strategies designed to prevent relapse to addictive behaviors, including cocaine use, heavy alcohol use, overeating, and tobacco smoking. Common among the varieties of CBT strategies is the primary focus on maintenance in the habit change process, i.e., preventing occurrence of initial lapses after one has embarked on a program of habit change, and/or preventing any lapse from escalating into a total relapse.[141] The Matrix Model of Relapse Prevention Counseling[142] has been used by NIDA DPMC in several clinical trials evaluating medications for methamphetamine dependence. The efficacy of this Model in the treatment of methamphetamine dependence has been demonstrated.[140] Overall, CBT is a feasible strategy that is generally well accepted by patients seeking treatment, and will be used in this study.

5   STUDY OBJECTIVES

5.1   Primary Objective

The primary objective of this study is to assess the efficacy of bupropion in reducing methamphetamine use in subjects with methamphetamine dependence who report using methamphetamine 29 or less days during the 30 day prior to signing consent. It is hypothesized that bupropion, compared to placebo, will be associated with an increase in the proportion of subjects who achieve abstinence (confirmed by at least two methamphetamine-negative urines and no methamphetamine-positive urines) each week during the last two weeks (Weeks 11 and 12) for non-daily users as the primary outcome.

5.2   Secondary Objectives

Secondary objectives include but are not limited to:

1. Assessing the efficacy of bupropion in reducing methamphetamine use in subjects with methamphetamine dependence who report using methamphetamine 18 or less days during the 30 day prior to signing consent.

2. Determining the safety of bupropion in the study population.

3. Assessing the efficacy of bupropion in other measures of success in the reduction of methamphetamine use determined by methamphetamine qualitative and quantitative urinalysis, self-report of use, and combinations of the two.

4. Assessing the efficacy of bupropion in the reduction in the severity of methamphetamine dependence (assessed by ASI-Lite and self and observer scored CGI), craving (assessed by BSCS), ADHD symptoms, (assessed by AISRS scores), and severity of depression (assessed by HAM-D) as compared to placebo control.

5. Assessing the rates of continued abstinence 4-weeks after completing the investigational product dosing period.

6. Assessing the effects on HIV risk taking behaviors.

7. Assessing the effects of bupropion on study retention.

8. Evaluating genetic variance and change in expression profiles on clinical outcomes including efficacy and safety.

9. Assessing the association of BDNF levels with clinical success and the influence of bupropion on BDNF levels.

6 IND HOLDER

This study will be conducted under an IND held by NIDA.

7 STUDY SITES

The trial will be conducted as a multi-center study at 5 or more clinical sites.

8 STUDY DESIGN

This is a double-blind, placebo-controlled, parallel-group design study in which, after screening and a 2-week baseline period, 200 participants will be randomly assigned to either receive placebo or bupropion (100 subjects per group) daily for 12 weeks, with a follow-up assessment 4 weeks after completion of study interventions. Adaptive randomization will be used to balance study intervention groups based on site, methamphetamine use (18 or less *versus* 19-29 days out of 30 days prior to signing consent), severity of depression symptoms (HAM-D score ≤ 12 *versus* >12), and presence of Adult ADHD.

9 SUBJECT SELECTION

Target randomization includes 200 males and females with methamphetamine dependence (approximately 100 subjects per group). Entry into this study is open to both men and women and to all racial and ethnic subgroups. An attempt will be made to randomize at least 30% females. Potential study subjects will be recruited from a variety of sources. The primary source will be individuals seeking treatment for methamphetamine dependence via referrals from local treatment providers and word of mouth from subjects already participating in the trial. Additional individuals will be recruited from the community by means of advertising in local media. Recruitment advertisements will be approved by NIDA and then by each site's Institutional Review Board (IRB).

9.1   Inclusion Criteria

Participants <u>must</u>:

1. Be males and/or females, between 18 and 65 years-of-age, inclusive.

2. Have a DSM-IV diagnosis of methamphetamine dependence as determined by MINI.

3. Have at least 1 amphetamine or methamphetamine positive urine specimen (> 500 ng/mL) after the start of screening and before randomization, or provide collateral information to verify recent use if a positive urine sample can't be obtained.

4. Report using methamphetamine for 29 or less days during the 30 day period prior to signing consent using the Timeline Follow-back method.

5. Be willing and able to comply with study procedures.

6. Be able to verbalize understanding of consent forms, able to provide written informed consent for both the main study and genetics study, and verbalize willingness to complete study procedures.

7. Be seeking treatment for methamphetamine dependence.

8. If female, have a negative pregnancy test during screening and on the first day of investigational product administration and agree to use of one of the following methods of birth control:

    a. prescription oral contraceptive
    b. contraceptive patch
    c. barrier (diaphragm or condom) with spermicide
    d. intrauterine progesterone or non-hormonal contraceptive system
    e. levonorgestrel implant
    f. medroxyprogesterone acetate contraceptive injection
    g. complete abstinence from sexual intercourse and agree to use another method should sexual activity commence
    h. hormonal vaginal contraceptive ring
    i. contraceptive sponge
    j. surgical sterilization
    k. partner is surgically sterile
    l. be post menopausal for one year

9.2   Exclusion Criteria

Participants <u>must not</u>:

1.   Have current dependence, defined by DSM-IV criteria, on any psychoactive substance (e.g., opioids) other than methamphetamine, nicotine, alcohol or marijuana or have physiological

dependence on alcohol or a sedative-hypnotic (e.g., a benzodiazepine) requiring medical detoxification.

2. Have a current or past history of seizure disorder, including alcohol- or stimulant-related seizure, or significant family history of idiopathic seizure disorder.

3. Currently be using drugs that lower seizure threshold.

4. Have a history of head trauma that resulted in neurological sequelae (e.g., loss of consciousness greater than 5 minutes, or that required hospitalization).

5. Have psychiatric disorders, such as major current depression, psychosis, bipolar disorder, organic brain disorder, or dementia as assessed by the MINI interview, or medical disorder, any of which require an excluded medication (e.g., antidepressant, neuroleptic, systemic corticosteroid, xanthine) or which would make medication compliance difficult. Have had electroconvulsive therapy within the past 90 days before screening.

6. Have a current suicidal ideation/plan as assessed by the MINI interview or HAM-D question #3. Current is identified as within the past 30 days.

7. Have a current or past history of anorexia nervosa or bulimia disorder.

8. Have serious medical illnesses or neurological disorders including, but not limited to, uncontrolled hypertension (See NIDA Guidelines on Hypertension in the Operations Manual, stage 1 hypertension allowed, but not stage 2 hypertension), significant heart disease (including myocardial infarction within one year of enrollment), angina, hepatic or renal disorders, renal insufficiency (plasma creatinine > 1.7 mg/dL), Parkinson's disease, active syphilis that has not been treated or refuse treatment for syphilis (see note), or have had therapy with any opiate-substitutes (methadone, LAAM, buprenorphine) within 2 months of enrollment, or any serious, potentially life-threatening or progressive medical illness other than addiction that may compromise subject safety or study conduct. Any ECG/cardiovascular abnormality (e.g., QTc interval prolongation > 450 milliseconds in men or 480 milliseconds in women), which in the judgment of the investigator is clinically significant.

9. Have diabetes with unstable control of blood glucose and have any incidence of hypoglycemia in the past year before screening.

10. Be mandated by the court to obtain treatment for methamphetamine-dependence where such mandate required the results of urine toxicology tests to be reported to the court.

11. In the opinion of the investigator, be expected to fail to complete the study protocol due to probable incarceration or relocation from the clinic area.

12. Be undergoing HIV treatment with antiviral and/or non-antiviral therapy since these drugs may increase the bupropion levels. The following list of HIV treatment/medications may be

used: Norvir, Reyataz, Truvada, Videx, Viread, Androgel and Trizovir. Note: Any HIV medication not on this list should be approved by the medical monitor.

13. Have known or suspected hypersensitivity to bupropion.

14. Be using bupropion or any medication that could interact adversely with bupropion, within the following times of beginning of administration of bupropion based on the longest time interval of A, B, and C, below or as otherwise specified:

    A) Five half lives of other medication or active metabolite(s), whichever is longer;
    B) Two weeks; or
    C) Interval recommended by other medication's product labeling.

Medications that fall into this category include:

    a. Bupropion (Wellbutrin®, Zyban®) used during the past 30 days
    b. All antidepressants
    c. Neuroleptics
    d. Systemic corticosteroids
    e. Xanthines, i.e., theophilline, theophilline sodium glycinate and aminophylline
    f. Antiretrovirals including nelfinavir, and efavirenz
    g. Tramadol (Ultram, Ultram Extended Release)

15. Have participated in any experimental study within 8 weeks (the nature of excluded studies may be discussed with NIDA investigators).

16. Be pregnant or breast feeding.

17. Have clinically significant laboratory values (outside of normal limits), in the judgment of the investigator.

18. Have liver function tests greater than 3 times the upper limit of normal.

19. Have active tuberculosis (positive tuberculin test and confirmatory diagnostic chest x-ray).

20. Have a diagnosis of adult (i.e., 21 years or older) asthma, or chronic obstructive pulmonary disease (COPD), including those with a history of acute asthma within the past two years, and those with current or recent (past 3 months) treatment with inhaled or oral beta-agonist or steroid therapy (because of potential serious adverse interactions with methamphetamine), or have an $FEV_1 < 70$ %.

Notes on inclusion/exclusion criterion: Although AIDS is an exclusion criteria, a positive antibody titer to HIV is not. If subjects ask about HIV testing during screening, they will be referred to an appropriate clinic that offers this service.

Prospective subjects who are positive for syphilis by the RPR test will have a fluorescent treponemal antibody absorbent assay (FTP-abs) or microhemagglutinin assay-Treponema pallidum (MHA-TP) confirmatory test performed. If this test is positive, prospective subjects must be treated for syphilis. If the prospective subject can provide evidence that they have been previously treated for syphilis or undergoes treatment for syphilis, they can be enrolled upon providing proof of successful treatment for syphilis.

The infectious disease panel for hepatitis and tuberculosis is performed as an aid to determine if the prospective subject has active hepatitis or tuberculosis. Either will exclude the prospective subject from participation according to exclusion criterion number 8 (serious medical illnesses) or number 19 (have active tuberculosis). All subjects who test positive for tuberculin PPD will be referred and scheduled to have a chest X-ray. Those that do not actually have tuberculosis will not be excluded from the study. Those that show a positive chest X-ray for tuberculosis will be excluded from the study. Prospective subjects who test positive for hepatitis will be evaluated by the site investigator for eligibility. Those subjects will be excluded if they have acute hepatitis or chronic active hepatitis, based on symptoms serology, and/or abnormal liver functions. Subjects who test positive for hepatitis will be referred for treatment as well as those who show positive chest X-ray for tuberculosis.

If any test results are positive subject will be notified of positive and confirmatory test results and will be referred to treatment.

Methamphetamine induced psychosis does not exclude a subject from the study, however the presence of current psychotic symptoms will exclude a subject from the study until clinically stabilized.

10 INVESTIGATIONAL PRODUCTS

10.1 Bupropion SR

Bupropion SR tablets, 150 mg, and matching placebo will be supplied by Murty Pharmaceuticals, Inc., (Lexington, KY) under a contract with NIDA. GSK's Zyban (bupropion) SR tablets, 150 mg, will be film-coated by Murty Pharmaceuticals to mask information on tablet surface. Matching placebo will be manufactured by Murty Pharmaceuticals. Data show that the film coating has negligible effect on the dissolution of Zyban SR tablets.

Bupropion's chemical name is (±)-1- (3-chlorophenyl)-2-[(1,1-dimethylethyl)amino]-1-propanone hydrochloride and has a molecular weight of 276.2. The structural formula is:
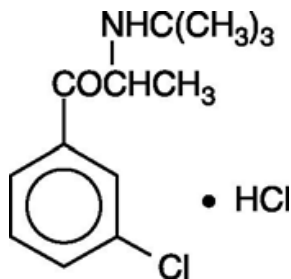
10.2 Placebo Control

Identically appearing placebo control tablets will be prepared by Murty Pharmaceuticals.

10.3 Dispensing Investigational Products

Murty Pharmaceuticals will distribute the investigational products packaged in HDPE bottles with child-resistant closure as a 10-day supply to investigators or designated Research Pharmacists at the clinical sites for dispensing to subjects. Used and unused investigational products will be collected and inventoried each week and an investigational product compliance CRF will be completed.

Investigational products will be dispensed to subjects once per week at the first clinic visit of the week. Investigational products will be distributed directly to the subject by the investigative staff depending upon local site procedures. The subject will be thoroughly instructed on how to administer investigational products.

Subjects will be instructed to store investigational products at room temperature without exposure to direct sunlight. Subjects will be instructed to consume the morning dose between 8:00 and 10:00 a.m. and the evening dose between 4:00 and 6:00 p.m. The exact time of the morning and evening doses may vary across patients depending on their schedules, but should be maintained constant for a particular individual. Subjects will be asked to refrain from taking the morning dose on the day during Week 6 and Week 12 that they are scheduled for a blood draw for bupropion levels. At these visits, subjects will be administered their daily dose in the clinic after blood collection. Administration of the daily dose in divided doses of maximum 150 mg twice daily, at least 8 hours apart, should minimize the risk of seizures. Unused investigational products will be collected and inventoried each week.

10.4 Labeling

Bottles of investigational products will be labeled by Murty Pharmaceuticals to include the following required statement – Caution: New Drug – limited by federal law to investigational use. Bottles will be distributed to investigational sites. The following information will be completed when a subject is assigned to a study group: subject's study identification number and site code. In addition, stickers will be provided with the words "Study Week X", where X is the study week number (i.e., 1 through 12), and date dispensed.

The label will also include a warning indicating that the investigational product should be kept out of children's reach. Special emergency wallet cards will be prepared for each site indicating the local Principal Investigator's name and 24-hour emergency telephone number. The study subject should be instructed to carry these cards with them at all times.

10.5 Storage

Investigational products will be stored at room temperature without exposure to direct sunlight in a secure location in the distributing pharmacy or at each investigator's facility.

10.6 Record of Administration

Accurate recording of all investigational product dispensing/administration will be made in the appropriate section of the CRF. On the first clinic visit of each week, subjects will be asked to return the bottle and all unused investigational product. Unused investigational product will be inventoried for discrepancies. Subjects who have not been taking their tablets regularly will be encouraged to do so in the future. New, unused investigational products (a 10-day supply) will then be dispensed to that subject. On each and all clinic visits (i.e. 3 times per week), self-reports of investigational product use since the last clinic visit will be recorded.

10.7 Used/Unused Investigational Products

During the study, all investigational products not used by the subject must be returned to the investigator for assessment of subject compliance. At the end of the study, all unused investigational products must be inventoried. If any investigational product is lost or damaged, its disposition should be documented. Unused investigational products will be returned to Murty Pharmaceuticals when indicated by NIDA or KAI and retained at Murty Pharmaceuticals until the end of the study.

11 INTERVENTIONS

11.1 Investigational Products

Subjects assigned to receive bupropion hydrochloride SR will receive 150 mg every day for the first 3 days of dosing which will be increased to 300 mg daily (one tablet twice a day) for the duration of dosing period until the final dose taper. The dose will be tapered to 150 mg every day for 3 days until termination or the last scheduled day of the 12-week intervention period. Subjects will be informed that they must taper the dose for three days before stopping the investigational products completely in the event that they decide to terminate from the study prematurely. Subjects assigned to the placebo group will receive matched placebo on the same schedule as the bupropion group. Subjects will be instructed to take investigational products at least 8 hours apart when taking investigational products twice daily and not to take two tablets at once if a dose is missed. During the last 3 days of study interventions during Week 12 or before termination, subjects' investigational products will be tapered down to 150 mg (or equivalent placebo) each day by taking only one tablet per day.

11.2 Cognitive Behavioral Therapy

All subjects will be offered and encouraged to attend two 60 minutes early recovery skills group sessions per week after signing consent but before randomization. After randomization, the CBT program will consist of thrice weekly, 90-minute group sessions through the 12-week investigational product dosing period of the trial with once weekly sessions during the 4-weeks of follow-up. In order to help potential participants to stop methamphetamine use, they will be introduced to the counselors and scheduled to attend two 60-minute early recovery skills groups each week after signing informed consent up until randomization. Topics covered in this early recovery skills group include: Getting Rid of Paraphernalia; Triggers; Introduction to 12-Step Groups; and Brief Information on HIV. Concepts presented in these sessions include the following: (1) self-monitoring and relapse analysis; (2) identification of "triggers" and cognitive

strategies for coping with them; (3) teaching of problem solving skills; (4) education about methamphetamine and methamphetamine dependence; (5) education about HIV and reducing the risk of HIV transmission; and, (6) promotion of prosocial activities. The content of these group topics is prearranged and sequenced using a manualized format. This is a feasible treatment that is known to be well accepted by subjects and it represents an appropriate, ethically defensible standard treatment condition to serve as the "platform" for the medication trial. Staff members who provide CBT counseling will have attended training in the use of these materials. The CBT specialist will have a minimum of a master's degree (or equivalent). To ensure that the integrity of these sessions is maintained, some of the sessions may be audiotaped. Refer to the Operations Manual for further instructions on the quality assurance process. Our experience is that these sessions are valued by subjects and attendance is excellent. Thus, psychosocial involvement is seen as a standard or platform for the proposed pharmacotherapy evaluation.

12 STUDY PROCEDURES

12.1 Subject Recruitment

Interested subjects who are seeking treatment and are available to come to the clinic for 18-to-24 weeks will meet with the investigator or designated investigational staff and receive an explanation of the study purpose and requirements. If still interested after receiving an explanation of the study, the subject will be given an opportunity to review, inquire about, and sign the informed consent form. A separate consent form will be used for the genetics analysis part of the study. Recruitment strategies vary across each site based on their local population, however standard tactics will be used (i.e., flyers, newspaper ads, radio ads). Local IRBs and NIDA will approve all advertising materials used for subject recruitment. Once the subject signs the consent form, s/he will be considered enrolled in the study.

12.2 Screening/Baseline Assessments

Screening and baseline assessments will be conducted as shown in **Table 1**. Screening and baseline assessments may be performed concurrently. A single blood draw for genetic samples will be performed during the screening period for those subjects who consented to the genetics part of the study. This one draw will result in the collection of one tube of blood for genomic DNA analysis and two tubes of blood for RNA analysis. Two additional tubes of blood will be collected for baseline plasma bupropion and BDNF levels determination during screening. The blood for genomic DNA analysis will also provide data to be used in conjunction with genetic analysis of methamphetamine dependence, even if the subject is ultimately found ineligible to participate in the main study. Data that will be used in the genomic analyses include: demographic data, medical and substance use history (timeline followback, quantity frequency interview, methamphetamine urine levels), family history, and diagnostic information obtained by the MINI. Detailed information about what data will be used in the genetic part of the study is enumerated in a separate Genetics Informed Consent Form.

Screening/baseline assessments will occur over a consecutive 14-28 day period. A potential subject must complete the minimum set of baseline measures over a consecutive 14-day period to be eligible for the study. If a subject fails to satisfy eligibility criteria during the screening and baseline periods, s/he may be re-screened. This decision will be made on a case-by-case basis.

Baseline measures over the consecutive 14-day period include collection of at least four urine specimens prior to randomization and the accompanying other baseline repeated measures. Demographic data and the reason that any subject did not meet eligibility criteria must be recorded for anyone who consented to screening even if the individual is a screening failure.

12.3 Subject Randomization

Adaptive randomization using an interactive voice randomization system (IVRS) will be used to balance study intervention groups within each clinical site based on methamphetamine use (18 or less *versus* 19-29 days out of 30 days prior to signing consent), severity of depression symptoms (HAM-D score ≤ 12 *versus* >12), and presence of adult ADHD. The procedure allocates group assignment based on the assignments and prognostic variable levels for all previously randomized subjects. A new subject will be randomized with a "biased coin" procedure that uses randomization probabilities, favoring the treatment with the deficit enrollment, to improve the balance on group assignment.[143]

When ready to randomize a subject, designated site staff will call the IVRS number and enter the information regarding each randomization variable presented above over the phone. These descriptors can then be used to randomize subjects into study intervention groups. Site staff enters relevant data using either the phone key pad or simple voice commands. The IVRS repeats the users' responses to verify entered data before saving and assigning an intervention group. The system allows the user to back up or re-enter data at any point prior to the intervention group assignment. The information is not finalized until the user reviews the entered data. Once the user has approved the information, the randomization algorithm is activated and an intervention group is assigned. All users are trained on the IVRS as part of the study training plan.

All randomization data are stored on the KAI network on a secure drive that is only accessible to a designated KAI network support staff member and the programmer responsible for programming the IVRS system. There is a physical firewall in place to prevent access to the KAI network via the Web. This limited access to randomization data is done to prevent inadvertent or unauthorized breaking of or tampering with the study blind.

12.4 Intervention Phase

At the first clinic visit, subjects will be given instructions on how to administer the investigative product, bupropion or placebo, and will be given a ten-day supply. This is a double-blind study in which neither the subject nor the site staff will know if the subject is receiving bupropion or placebo. The subject will be given the first dose of the investigational product in the clinic regardless of the time and will be observed for one hour to monitor for immediate adverse symptoms.

Subjects will be scheduled for CBT and assessment visits three times per week usually on a Monday, Wednesday, and Friday for 12 weeks. Two consecutive days may be scheduled around holidays or other schedule conflicts. Clinical evaluations are described in detail in Section 13.

12.5 Completion Interview

After the completion of dosing, (as soon as possible after the last dose of investigational product is taken during Study Week 12) or at the time of premature study discharge, subjects will be asked to complete the study completion interview. Vital signs, physical examination, a 12-lead ECG, pregnancy test and clinical laboratory studies (blood chemistry, hematology, and urinalysis) will be performed. The ASI-Lite, HRBS, CGI-O, CGI-S, AISRS, and End of Trial form (if they complete the study before Week 12) will be completed in addition to the scheduled weekly assessments. Methamphetamine and creatinine and other drug urine tests, alcohol breathalyzer, if necessary, according to institutional policies, and SUR will be completed.

12.6 Drop-Out Prevention

Subjects will be encouraged to come for CBT sessions and for the clinical evaluations as described in this protocol. To minimize missed sessions, they will be reimbursed for transportation and time spent in completing study assessments. It will be emphasized to subjects during screening that even if they have a relapse they should come to all scheduled appointments. They will be discouraged from using methamphetamine, but there will be no penalty for relapsing or for missed sessions.

Subjects will be encouraged to complete study visits, assessments, and CBT sessions, even if they are unable to tolerate the investigational products. If a subject decides to drop out of the study prior to week 12, s/he will be asked to complete all final assessments (termination) at the time of drop out. If a subject wishes to stop taking the investigational product but to continue to participate in CBT sessions, s/he will continue to have all scheduled assessments according to the protocol and will complete the study at Week 16.

A subject will be considered a drop out, if the subject misses six consecutive visits during the 12 weeks of study interventions. However, s/he will be asked to complete all termination assessments at the time of drop out and will be compensated for completing these assessments.

Subjects are free to discontinue the study and to refuse to participate in any of the procedures. If a subject drops out without making their intentions known, staff will attempt to contact them by telephone or written correspondence. Once the subject has been contacted and expresses their decision to discontinue from further participation in the study, the research staff will cease to try to make further contact.

12.7   Follow-up (Weeks 13 - 16)

Subjects will be asked to return to the clinic once per week for 4-weeks after the completion of the study intervention phase of the trial. Subjects will receive CBT, and will be asked to provide a urine specimen for methamphetamine/creatinine and urine drug screen, to provide a self-report for use of methamphetamine, cocaine, opiates, alcohol, cigarette smoking, and marijuana, and will be asked about AEs and concomitant medication use. The subject will complete the follow-up form at Week 16. If it is not possible to arrange for the subject to return to the clinic, then they should be telephoned and asked to provide a current self-reported methamphetamine and other drug use and other current treatment for drug or alcohol abuse. Concomitant medications

and AEs will be assessed over the telephone as well as the follow-up form. If a subject cannot be contacted directly, attempts will be made to reach the individual(s) previously identified by the subject as a contact source.

12.8   Maintaining and Breaking Study Blind

The decision to break the study blind for an individual subject lies with the site principal investigator and project principal investigator or with the NIDA medical monitor, but should be resorted to only in cases of life-threatening emergency when knowledge of the investigational product identity will influence clinical management. KAI will develop an unblinding procedure for the study and ensure that emergency unblinding is possible 24 hours a day, seven days a week. This procedure could include leaving treatment information in a sealed envelope with a medical monitor, pharmacist or other designated individual at the participating center.

12.9   Safety Procedures

Subjects judged by the site principal investigator and/or study physician at any point to be a danger to self or others or who are judged to be in grave danger due to continued investigational product use and/or to extreme psychiatric problems will be discontinued from the study and connected with an appropriate treatment agency.

12.10   Subject Compensation

Subjects will be reimbursed for travel expenses, for providing data, and for time contributed to this research study in accordance with local site's IRB policies.

12.11   Subject Confidentiality

To maintain subject confidentiality, all laboratory specimens, eCRFs, reports, and other records will be identified by a coded number and name code only. Research and clinical records will be stored separately in a secure location and only the investigative staff will have access to the records. Subject information will not be released without the written permission, except as necessary for monitoring by the FDA, NIDA, its designees, or other regulatory agencies. Upon approval of the study by the site IRB, an application will be filed with NIDA for a certificate of confidentiality.

12.12   Study Termination

12.12.1   Subject Termination
An investigator may terminate a subject if s/he deems it clinically appropriate or for any of the following reasons: (1) significant side effects from investigational products, (2) serious or unexpected AEs, (3) failure to comply with the study protocol, (4) protocol violation, (5) positive pregnancy test, (6) serious intercurrent illness or a benzodiazepine or barbiturate medication use (see below), and (7) the subject becomes a danger to self or others. Persistent alcohol abuse will be assessed by the PI or study physician and after the third warning and consultation with NIDA, the subject may be terminated from the study.

Benzodiazepine and Barbiturate Abuse. If a subject is found to be persistently abusing a benzodiazepine or barbiturate medication while in the study, they will likewise be given a warning by the site principal investigator or study physician to stop using these drugs, and continued abuse of a benzodiazepine or barbiturate beyond 3 weeks after the date the warning was given may result in discontinuation of bupropion. A three-week interval is warranted due to the long elimination time of some benzodiazepines. If a subject is prescribed a benzodiazepine or barbiturate for legitimate medical purposes, subjects will be warned that any abrupt discontinuation of either medication is known to lower seizure threshold.

Failure to comply with protocol. A subject will no longer be eligible for study participation, if s/he is absent from the clinic for more than two consecutive weeks and does not provide any data. A subject's study participation will end if s/he fails to provide six consecutive urine samples. In any event, subjects will be contacted 4 weeks after completing study interventions to schedule a follow-up interview.

Voluntary withdrawal. A subject may withdraw from the study anytime s/he wishes. In the event a subject is discontinued from receiving the investigational products, s/he will be allowed to continue the CBT with the approval of the site principal investigator.

Any subject, who discontinues prematurely, regardless of the reason, will be requested to return for a final visit to perform the necessary procedures and obtain data for the final assessments to be completed at the end of the study intervention phase as well as 4 weeks later for the follow-up interview.

If at any time during the course of the study, psychiatric symptoms are so severe as to require medication outside of the protocol and/or hospitalization, the participant should be terminated from the study protocol and treated clinically. Study participants withdrawn from the protocol secondary to a medical or psychiatric concern will be referred for appropriate treatment. Subjects will be asked to sign a general consent for the release of information to the referred health care. Study staff may request transportation for emergency treatment of a subject if medically appropriate (e.g., for acutely psychotic or suicidal subjects).

All study participants will be advised to carry an emergency wallet card that identifies them as a participant in a clinical research study. The card will provide the name and phone number of the site principal investigator (physician) at the site who can be contacted in the event of an emergency. If possible, the card should also instruct the non-study physician rendering emergency care to provide information to the study physician with regards to that care.

12.12.2   Trial Discontinuation
NIDA has the right to discontinue the investigation at any time.

12.13 Concomitant Medications

Any medications (including prescription, over-the-counter, herbal supplements and health store products) to be taken during the study must be approved by the site principal investigator. Medications that should not be taken at any time during the study include:

- All antidepressants
- Neuroleptics
- Systemic corticosteroids
- Xanthines (i.e., theophilline, theophilline sodium glycinate and aminophylline)
- Medications that interfere with methamphetamine detection in urine samples (e.g. ephedrine and pseudoephedrine)
- Bupropion containing medications (i.e., Wellbutrin®, Zyban®)
- The antiretrovirals nelfinavir, and efavirenz
- Tramadol (Ultram, Ultram Extended Release)

13 CLINICAL EVALUATIONS

Clinical evaluations will be administered according to the schedule in **Table 1**. Assessments were chosen to minimize the research burden, yet collect adequate data to address study hypotheses.

13.1 SCREENING ASSESSMENTS

Prior to randomization into the study, subjects will be screened to determine if they meet eligibility requirements. In addition, certain baseline assessments that are part of eligibility determinations will also provide physiological, psychological, and disease status information prior to investigational product administration. Screening and baseline assessments may be performed concurrently; however, baseline assessments will include only those acquired during a 14-day consecutive period prior to randomization. An End of Trial eCRF must be completed if a subject fails to meet eligibility requirements or discontinues screening before eligibility is established.

1. Informed consent
2. Locator form
3. Demographics
4. Timeline follow-back for methamphetamine use in the prior 30 days
5. Quantity frequency interview
6. Psychiatric evaluation and MINI evaluation for DSM-IV diagnosis of methamphetamine dependence and Axis-I disorders
7. Adult ADHD interview (ACDS)
8. Medical history and Family History Questionnaire
9. Prior medications for the 30 days prior to informed consent
10. Infectious disease serology/Syphilis test
11. Alcohol breathalyzer
12. Physical exam
13. Vital signs
14. Hematology and blood chemistries
15. Blood for DNA analysis (for all subjects who consent to genetics part of study)
16. Blood for RNA analysis (for all subjects who consent to genetics part of study)
17. Blood for plasma BDNF levels (for all subjects)

18. Blood for baseline plasma bupropion levels (for all subjects but will only be analyzed for subjects who are randomized onto the study
19. Medical Urinalysis
20. Pregnancy Test
21. ECG
22. AEs at each visit
23. Concomitant medication use at each visit
24. ASI-Lite
25. HAM-D (performed weekly during screening/baseline period)
26. Urine drug screen – onsite test device (3 times per week)
27. Urine Methamphetamine/Creatinine for Central Lab (3 times per week) Note: Assay for methamphetamine and creatinine as well as the urine toxicology screen will be performed only for urine samples collected from the subjects who are randomized into the study.
28. SUR (3 times per week)
29. CBT Compliance (two 60-minute sessions weekly, to be checked at every visit and verified with the CBT attendance log)
30. Eligibility Checklist (initial evaluation)

Assessment	Screening	Baseline	Intervention Phase													Follow-up			
Study Week	-4 to -1	-2 to -1	1	2	3	4	5	6	7	8	9	10	11	12/Term	13	14	15	16
Screening																			
Informed consent	X																		
Locator Form	X																		
Demographics	X																		
Timeline follow-back (30 days)	X																		
Quantity Frequency Interview	X																		
MINI	X																		
ACDS	X																		
Medical and family[a] history	X																		
Prior medications	X																		
Infectious disease panel/RPR	X																		
Alcohol breathalyzer[b]	X	3 X/week	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	X	X	X	X
Safety																			
Physical exam	X													X[l]				
Vital signs	X		X	X	X	X	X	X	X	X	X	X	X	X[l]				
Hematology	X													X[l]				
Blood chemistries	X													X[l]				
Medical urinalysis	X													X[l]				
Pregnancy test[c]	X		X			X				X				X[l]				
ECG	X													X[l]				
Adverse events[d]	3X/week	3X/week	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	X	X	X	X
Concomitant medications[d]	3X/week	3X/week	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	X	X	X	X
Eligibility Checklist[e]	X	X																
Efficacy																			
ASI-Lite[f]	X	X												X[l]				
AISRS		X				X				X				X[l]				
HAM-D	weekly	weekly		X		X		X		X		X		X[l]				
BSCS		weekly	X	X	X	X	X	X	X	X	X	X	X	X[l]				
CGI-S		weekly	X	X	X	X	X	X	X	X	X	X	X	X[l]				
CGI-O		weekly	X	X	X	X	X	X	X	X	X	X	X	X[l]				
HRBS		X												X[l]				
SUR	3X/week	3X/week	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	X	X	X	X
Urine methamphetamine/creatinine[g]	3X/week	3X/week	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	X	X	X	X

Assessment	Screening	Baseline	Intervention Phase													Follow-up			
Study Week	-4 to -1	-2 to -1	1	2	3	4	5	6	7	8	9	10	11	12/Term	13	14	15	16
Urine bupropion/hydroxybupropion[n]			3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X				
Blood for DNA (genetics consent)	X																	
Blood for RNA[h] (genetics consent)	X							X						X[l]				
Bupropion Plasma Levels[i]	X							X						X[l]				
BDNF Plasma Levels	X							X						X[l]				
Urine drug screen – onsite test device	3X/week	3X/week																
CBT compliance	X[k]	X[k]	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	X[m]	X[m]	X[m]	X[m]
Investigational Product compliance[j]			X	X	X	X	X	X	X	X	X	X	X	X[l]				
End of Trial form	X – if screen fail																	X
Follow-up questionnaire																		X

[a] The family history questionnaire will only be done on subjects who consent to the genetics testing even if they are considered a screen failure for the bupropion/methamphetamine study.

[b] An alcohol breathalyzer test will be performed in accordance with institutional policy and state law.

[c] The pregnancy test on Day 1 must be performed prior to randomization and administration of the first dose of investigational product.

[d] These assessments are performed after the first screening visit

[e] Eligibility is checked during screening and at baseline visits as data become available such that if a subject does not meet inclusion or exclusion criteria, the subject is no longer screened.

[f] The ASI-Lite will be performed during screening; however, if the entire screening/baseline period takes longer than 14 days, the ASI must be repeated such that the it is collected during baseline.

[g] Assay for methamphetamine and creatinine as well as the urine toxicology screen will be performed only for urine samples collected from the subjects who are randomized into the study.

[h] Blood for RNA analysis should be collected at the same time of day at every collection time point to control for circadian rhythm effects on expression. One RNA tube will be collected during screening/baseline and at all other time points during the intervention phase of the study.

[i] During the intervention phase, blood for bupropion levels will be taken before the daily dose.

[j] Investigational product compliance is reviewed at every clinic visit with the subject by self report but the pill count is only done weekly.

[k] Two 60 minute early recovery skills group sessions given weekly until randomization occurs. Compliance should be checked at each visit and verified with the CBT attendance log.

[l] These assessments are performed at the last visit of Week 12 or at earlier termination.

[m] CBT sessions are performed once a week during the follow-up period.

[n] Bupropion/hydroxybupropion will be analyzed after study closure only in those assigned to a bupropion treatment group as a surrogate marker for medication compliance.

13.2 BASELINE ASSESSMENTS

Baseline assessments to be completed over a 14-day consecutive period prior to randomization include the following:

1. The following will be obtained at each baseline visit (visits should be scheduled to occur three times a week for two weeks):
   a. Alcohol breathalyzer test (if necessary, according to institutional policies)
   b. AEs
   c. Concomitant medications
   d. Urine drug screen using an onsite testing device. Subjects must provide at least 4 urine specimens in a consecutive 14-day period with at least 2 of the specimens being collected during each of the two weeks. Ideally, 3 of the specimens will be obtained in one week and 3 in the next week. Also, no more than two specimens can be collected on consecutive days.
   e. Urine methamphetamine plus creatinine measurements. Note: Methamphetamine assay will be performed only for the urine samples collected for the subjects who are randomized on the study.
   f. CBT compliance (two 60-minute sessions weekly, to be checked every visit and verified with the CBT attendance log)

2. The following will be obtained weekly for two weeks:
   a. BSCS
   b. CGI-S
   c. CGI-O
   d. HAM-D
   e. Urine drug screen (all urine specimens will be sent to a central laboratory for analysis). This is only for subjects who are randomized on the study.

3. Daily report of methamphetamine, marijuana, nicotine, alcohol, opiates, and cocaine use will be recorded at each visit on a SUR CRF.

4. The following will be obtained once during baseline:
   a. ASI-Lite (If the ASI-Lite taken during screening is not during the baseline period, this assessment must be repeated during baseline. The reason for taking this during screening is to try to capture this data for all subjects for the genetics analysis, even if not enrolled in the study).
   b. AISRS
   c. HRBS

5. The Eligibility Checklist will be reviewed one final time at the end of the baseline period before randomization to ensure eligibility to participate in the study.

13.3 ASSESSMENTS DURING THE INTERVENTION PHASE

Over the 12-week period of study interventions, subjects will return to the clinic three times per week (ideally on Monday, Wednesday, and Friday). Assessments will be performed as follows:

On Day 1, Week 1 before dosing:

All women will have a pregnancy test on the day that first dose of investigational product is administered prior to the dose to confirm that the subject is not pregnant.

At each visit:

1. SUR
2. Urine methamphetamine and creatinine
3. Alcohol breathalyzer (if necessary, according to institutional policies)
4. AEs
5. Concomitant medications
6. Compliance with study interventions [investigational product dosing (by subject self report; pill counts are performed once per week) and CBT]

Once per week preferably at the first visit of each week (except at the end of Week 12):

1. Urine drug screen (urine specimen sent to central laboratory for analysis)
2. BSCS
3. CGI-S
4. CGI-O
5. Vital signs
6. Compliance with study interventions (pill count)

At Weeks 6 and 12, preferably at the first visit of the week:

1. Blood for RNA analysis
2. Blood for trough plasma bupropion levels (hold morning dose until blood sample obtained)
3. Blood for plasma BDNF levels

At Weeks 4 and 8, preferably at the first visit of the week, and Week 12 at the last visit of the week:

1. Pregnancy test
2. AISRS

At Weeks 2, 4, 6, 8, 10 and 12, preferably at the first visit of the week (except at the end of Week 12):

1. HAM-D

NOTE: Blood chemistries and hematologies will be performed during the intervention phase of the study if considered necessary by a study principal investigator.

13.4 ASSESSMENTS AT TERMINATION

At the end of Study Week 12 or if the subject discontinues prematurely, regardless of the reason (request that the subject return for final assessments), the following assessments will be performed:

1. If the subject discontinued prematurely, determine the reason for termination and complete the End of Trial form.

2. Physical exam
3. Vital signs
4. SUR
5. Urine methamphetamine and creatinine
6. Alcohol breathalyzer (if necessary, according to institutional policies)
7. AEs
8. Urine drug screen (urine specimen sent to central laboratory for analysis but only if a specimen was not already collected during that week)
9. BSCS
10. CGI-S
11. CGI-O
12. Hematology
13. Blood chemistries
14. Medical Urinalysis
15. Pregnancy Test
16. ASI-Lite
17. HRBS
18. HAM-D
19. AISRS
20. ECG
21. Concomitant medications
22. Compliance with study interventions (investigational product dosing and CBT)

13.5 ASSESSMENTS DURING FOLLOW-UPS (WEEKS 13 - 16)

Subjects will undergo the following assessments during follow-up visits (weekly):

1. Urine methamphetamine and creatinine
2. SUR (timeline followback for each week)
3. Alcohol breathalyzer (if necessary, according to institutional policies)
4. AEs
5. Concomitant medications
6. Compliance with CBT
7. Follow-up questionnaire (Week 16)
8. End of Trial form

In addition, at Week 13, if the subject has not already done so, s/he will turn in unused investigational products and complete final drug accountability.

13.6 Assessment Methods

General Comment Regarding Assessments: If the subject is unable to self-administer any assessment (e.g. physical handicap, poor reading skills) study personnel can assist by reading the questions out loud to the subject and/or marking the subject's response on the source document/CRF. However, study personnel are not to offer interpretations of the questions.

13.6.1   Adverse Events (AEs)

A research nurse, physician, or medically trained staff will assess AEs at all study visits starting at the time of signing informed consent. If an AE is reported that requires medical attention, it should be reported to a study physician immediately. The study physician will meet with the study staff once a week to review AEs recorded on all subjects, and the study physician may then meet any subjects for whom additional follow-up or AE assessment is indicated. The study physician will also assess the subjects for any medical or psychiatric adverse event. Both the nurse and physician will assess AEs by asking the subject "How have you been feeling since I last saw you?" The type of AE, severity of the AE and the relationship of the AE to the investigational product will be recorded on an AE CRF, according to the procedures described in Section 14.6.

13.6.2   Addiction Severity Index (ASI)-Lite CF Version

The ASI-Lite, 2000 version,[144] will be administered by a research staff member having at least a bachelor's degree in the social sciences or equivalent training and experience as determined by the site's investigator. The ASI-Lite is the interviewer's estimate of the severity of the subject's status in seven areas (medical, employment, drug use, alcohol use, legal, family/social, and psychological). Composite scores will be calculated according to the procedures described by McGahan *et al.*[145] and Carroll *et al.*[146] The Lite version is a shorter version of the ASI that still retains all questions used to calculate the ASI composite scores.

13.6.3 Adult ADHD Clinical Diagnostic Scale (ACDS)

An Adult ADHD Clinical Diagnostic Scale (ACDS) will be used to determine a diagnosis of adult ADHD at screening. The ACDS (V1.2) is a semi-structured interview, which documents current symptoms that contribute to the diagnosis of ADHD in an adult. It has 18 items that match the 18 symptom domains of ADHD noted in DSM-IV. There are suggested probes to establish the presence and extent of ADHD symptoms, as well as their severity and impact on subject functioning. The childhood component of the ACDS is an adaptation of the ADHD module of the Schedule for Affective Disorders and Schizophrenia for School Aged Children (KSADS-PL; Kaufman *et al.*[147]). Scoring is on a 4-point Likert scale: None, Mild, Moderate or Severe.

13.6.4 Adult ADHD Investigator Symptom Rating Scale (AISRS)

The AISRS consists of the 18 items and their probes from the ACDS, albeit, in a different order. It is used to take repeated measures of ADHD symptoms, in order to observe changes over time. A version of this instrument was used to test the reliability of ratings obtained from different, trained raters.[148] This assessment will be done four times, at baseline and Weeks 4, 8, and 12.

13.6.5   Blood Chemistries

Blood will be collected in serum separation vacutainer tubes and serum separated according to standard procedures. Quantitative analysis will be performed for the following analytes: sodium, potassium, chloride, carbon dioxide, glucose, creatinine, albumin, total protein, alanine aminotransferase (ALT/SGPT), aspartate aminotransferase (AST/SGOT), total bilirubin, lactate dehyrodrogenase (LDH), alkaline phosphatase (ALP), blood urea nitrogen (BUN), calcium and phosphorous. Blood chemistries will be performed at a local clinical laboratory. Laboratories

performing these assessments should be either directly regulated by CAP or CLIA or indirectly according to CLIA guidelines. The laboratory will need to provide a copy of current certification.

13.6.6 BDNF Plasma Levels
Blood will be collected in anticoagulant containing tubes and plasma separated according to standard procedures. Aliquots of the plasma will be frozen and shipped in batches to a central laboratory for analysis. The details of sample collection, labeling, storage, and shipment to the laboratory will be provided in a Study Procedures Manual.

13.6.7 Breathalyzer Test
The breathalyzer or breath alcohol test will be administered as necessary and in accordance with the site's local procedures if the subject presents at the clinic in an apparent intoxicated state.

13.6.8 Brief Substance Craving Scale (BSCS)
The BSCS is a self-administered assessment that asks the participant to rate his or her craving for methamphetamine. The BSCS used for this study is a modification of the State of Feelings and Cravings Questionnaire. If the participant is unable to self-administer this assessment (e.g. physical handicap, poor reading skills) study personnel can assist by reading the questions out loud to the participant and/or marking the participant's response on the source document/CRF. However, study personnel are not to offer interpretations of the questions.

13.6.9 Bupropion Plasma Levels
Blood will be collected in anticoagulant containing tubes and plasma separated according to standard procedures. Aliquots of the plasma will be frozen and shipped in batches to a central laboratory for analysis. The details of sample collection, labeling, storage, and shipment to the laboratory will be provided in a Study Procedures Manual. The time of blood collection will be recorded. In addition, the subject will be asked to provide the time that they took their last dose of bupropion/placebo before blood draw. During Weeks 6 and 12, the blood sample should be collected before the morning dose of investigational product to determine trough levels.

13.6.10 Clinical Global Impression-Observer (CGI-O)
The CGI-O requires the observer to rate the global severity of the subject's methamphetamine dependence symptoms and to rate the improvement of the subject's methamphetamine dependence symptoms since the beginning of the study. The severity of the subject's methamphetamine dependence is rated according to eight specific problem areas often associated with methamphetamine dependence.

13.6.11 Clinical Global Impression-Self (CGI-S)
The CGI-Self is a self-administered assessment that asks the subject to rate the global severity of his or her methamphetamine dependence symptoms and to rate the improvement of his or her methamphetamine dependence symptoms since the beginning of the study.

13.6.12 Concomitant Medications
All medications taken by the subject after consent during screening, while on study, and at the final follow-up assessment will be recorded on a Concomitant Medications form. The reported

medications will be reviewed by the site investigator/study physician for possible drug interactions.

13.6.13   ECG
Twelve-lead electrocardiograms will be performed according to standard procedures. The results will be reviewed by the investigator or study physician for interpretation. The investigator may consult a board-certified cardiologist, if necessary.

13.6.14   Eligibility Checklist
An Eligibility Checklist will be reviewed upon completion of screening and must be completed prior to randomization. This information will be used to determine whether the subject may be randomized to the study. This form will document final eligibility, date of first study day and, if applicable, the reason subject was not randomized to the study.

13.6.15   End of Trial Form
If the potential subject fails screening, complete the End of Trial form documenting the reason screen failure. In addition, during the study termination interview, all data relevant to the subject's withdrawal: reason for withdrawal; date of final visit; and study day of final visit will be collected.

13.6.16   Family History Questionnaire
A Family History Questionnaire that asks the subject about his/her family's history of substance abuse will be collected during screening for all subjects who consent to the genetics testing part of the study.

13.6.17   Follow-up Locator Form
A locator form will be used to assist in finding participants at follow-up. This form asks subjects to give consent for follow-up and to provide names, addresses, and phone numbers of several friends and family members. This information is essential and will be updated throughout the study as necessary.

13.6.18   Follow-up Questionnaire
The Follow-up Questionnaire will document the information collected at the Week 16 follow-up interview including if contact was made with the subject or documenting the subject's death. In addition, the form asks questions regarding the subject's drug use, and current treatment for drug and alcohol abuse.

13.6.19   Hematology
Blood will be collected in anticoagulant containing vacutainer tubes for hematologic assessments. Complete blood counts (CBC) with differentials and platelet count will be performed. Quantitative analyses for hemoglobin, hematocrit, red blood cells, platelets, total white blood cells, and percentage of neutrophils, lymphocytes, monocytes, eosinophils, basophils, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) will be performed. Analyses will be performed in a local clinical laboratory. Laboratories performing these assessments should be either directly regulated by the College of Pathologists (CAP) or the Clinical Laboratory Improvement Act of

1988 (CLIA) or indirectly according to CLIA guidelines. The laboratory will need to provide a copy of current certification.

13.6.20   *HIV Risk-Taking Behavior Scale (HRBS)*
The HRBS is assessed by interview of the subject's engagement in activities that increase the likelihood of contracting HIV. Study personnel are not to offer interpretations of the questions.

13.6.21   *Infectious Disease Panel/Syphilis Test*
Blood will be collected in serum separation vacutainer tubes and serum separated according to standard procedures. Qualitative analysis reporting positive/negative results will be performed for the following analytes: Hepatitis B surface antigen, Hepatitis B surface antibody, Hepatitis B core antibody, and Hepatitis C virus antibody. A purified protein derivative (PPD) skin test for tuberculosis will be performed and, if positive, a chest x-ray is required to assess active tuberculosis. If the subject reports that they have been previously positive for the PPD test, the PPD test will not be performed and a chest x-ray will be required. A rapid plasma reagin test (RPR) for syphilis will be performed. If positive, an FTA-abs or MHA-TP confirmatory test will be performed. These tests will be conducted at a local clinical laboratory.

13.6.22   *Hamilton Depression Rating Scale (HAM-D)*
The HAM-D is an interviewer-administered assessment of the subject's level of depression.[149] The HAM-D for this study includes three additional questions all associated with methamphetamine dependence (22. Helplessness, 23. Hopelessness, and 24. Worthlessness). The HAM-D will be administered by study research staff (i.e., project coordinator, research assistant) who will receive training on completing this measure.

13.6.23   *Intervention Compliance*
Compliance with study interventions will be monitored by recording the amount of investigational products taken each by the subject and will be recorded on a form weekly. The timeline followback method will be used to assist the subject in reporting of the amount of tablets taken between clinic visits. The timeline followback will be administered by the research staff three times a week and reviewed weekly by a physician. On each and all clinic visits (i.e. 3 times per week during the investigational product dosing period and once per week during the follow-up phase), self-reports of investigational product use since the last clinic visit will be recorded on the appropriate CRF. Compliance with CBT will be monitored by recording the length of time the subject spent in attendance at each therapy session and recorded on a form for each visit.

13.6.24   *Medical History*
To monitor the health of all study subjects, health profiles will be collected prior to participation in the study. A review of systems will be conducted by the site principal investigator/study physician or nurse practitioner to assure medical fitness. Female will also be asked about current method of birth control and will be counseled about using an approved form of birth control during the study.

13.6.25 Physical Exam

A physical exam of the oral cavity, head, eyes, ears nose and throat, cardiovascular system, lungs, abdomen (liver/spleen), extremities, skin, neuropsychiatric mental status and sensory/motor status, musculoskeletal system and general appearance should be performed. Height and weight will be recorded.

13.6.26 Prior Medications

All medications taken by the subject for the 30 days prior to screening will be documented on a Prior Medication form. The reported medications will be reviewed and approved by the site principal investigator/study physician.

13.6.27 Quantity Frequency Interview

Substance use histories will be determined by a structured interview with the Quantity Frequency Interview which quantifies the frequency and amount of substances of abuse used in the 30-days prior to screening and during the past year and overall lifetime use.

13.6.28 Mini-International Neuropsychiatric Interview (MINI)

The MINI is a short structured diagnostic interview, developed jointly by psychiatrists and clinicians in the United States and Europe, for DSM-IV and ICD-10 psychiatric disorders [150] With an administration time of approximately 15 minutes, it was designed to meet the need for a short but accurate structured psychiatric interview for multicenter clinical trials and epidemiology studies and to be used as a first step in outcome tracking in nonresearch clinical settings.

13.6.29 Urine Collection and Analyses

Urine will be collected for five types of analyses as follows:

1) Methamphetamine/amphetamine, creatinine, tetrahydrocannabinol, cocaine, opiates, and benzodiazepines analyses performed at a central laboratory.
2) Urine Toxicology Screen performed with a qualitative onsite test device for methamphetamine, cocaine, tetrahydrocannabinol, amphetamines, barbiturates, opiates and benzodiazepines.
3) Medical urinalysis performed at a local laboratory.
4) Pregnancy test.
5) Bupropion and metabolite (hydroxybupropion) analyses performed at a central lab.

Depending upon the assessment schedule, urine samples will be collected and aliquoted into the appropriate number of specimens. One specimen will be held frozen at the clinical site as a backup.

The others will be tested immediately or will be frozen or refrigerated as appropriate. Specimens will be collected and tested as follows:

Methamphetamine, Creatinine, Tetrahydrocannabinol, Cocaine, Amphetamines, Opiates, and Benzodiazepines Analysis. During the screening and baseline period of the study, urine will be collected 3 times per week (generally Monday, Wednesday, and Friday, barring holidays and

schedule conflicts). Urine samples will be tested using the onsite testing device. Subjects qualifying for entry into the study will be randomized. Upon randomization, the urine samples collected from subjects and stored during the screening/baseline period will then be sent to a central laboratory. The central laboratory will: a) perform qualitative analysis for the 1_{st} sample of the week for cocaine, tetrahydrocannabinol, methamphetamine/amphetamines, opiates, benzodiazepines and creatinine, b) perform qualitative analysis the 2_{nd} and 3_{rd} samples of the week for amphetamines/methamphetamine, creatinine , and c) perform quantitative analysis for methamphetamine and amphetamines for all the samples qualitatively screened positive for methamphetamine/amphetamines.

Note: The sites will store samples from screening/baseline until the subject is randomized, and once randomized all urine samples can be sent to the central laboratory for analysis. Urine samples will not be sent to the central laboratory if a subject is not randomized.

Following randomization, during the investigational product dosing period of the study and at each of the follow-up visits, urine will be collected 3 times per week (generally Monday, Wednesday, and Friday, barring holidays and schedule conflicts) and sent to a central laboratory. The central laboratory will: a) perform qualitative analysis for the 1_{st} sample collected each week for cocaine, methamphetamine/amphetamines, tetrahydrocannabinol, opiates, and benzodiazepines, b) screen all samples for amphetamines/methamphetamine, creatinine, and c) will quantitate amphetamines and methamphetamine for all samples, qualitatively screened positive for methamphetamine/amphetamines.

All specimens collected and screened positive by the central laboratory for methamphetamine/amphetamines will be subjected to methamphetamine quantitative analysis performed at central laboratory. The back-up sample retained at the site will be stored frozen until the NIDA data coordinating center has notified the site that it can be disposed.

Urine Toxicology Screen Using an Onsite Testing Device. During the screening/baseline period, urine will be collected 3 times per week (generally Monday, Wednesday, and Friday, barring holidays and schedule conflicts). After the backup aliquot and the central laboratory testing aliquot have been obtained, the sample will be analyzed using an on-site testing device (tetrahydrocannabinol, methamphetamine, cocaine, amphetamines, barbiturates, opiates and benzodiazepines). Samples positive for amphetamine/methamphetamine (the on-site testing device has a cutoff of greater than or equal to 500 ng/mL) will be considered as positive for methamphetamine for inclusion criteria purposes.

Medical Urinalysis. Urine will be collected and analyzed for color, appearance, specific gravity, pH, blood, protein, glucose, ketones, leukocytes, bilirubin and nitrites. Analysis will be conducted at a local laboratory.

Urine Pregnancy Test. An on-site qualitative urine pregnancy test that evaluates human β-chorionic gonadotropin will be used.

Bupropion and Metabolite (hydroxybupropion) Assays. Urine samples collected during the treatment period for the bupropion group will be assayed by a central lab for bupropion and its

metabolite, hydroxybupropion, using a liquid chromatography/mass spectrometry method. The limit of quantification will be 5 ng/mL for both bupropion and hydroxybupropion. The results will be used for the assessment of medication compliance and samples will be regarded as "positive" if bupropion and/or hydroxybupropion is present at or above the limit of quantification. For these assays, the central lab will utilize the same urine samples provided for assays of methamphetamine, creatinine, etc. (described above).

13.6.30 Genetic Analysis

Conduct of the following analyses will be dependent upon favorable efficacy findings. Microarray technology from Affymetric will be used to perform genotyping (on DNA samples) and expression analysis (on RNA samples). The GeneChip® Mapping 500K array set will be used for genotyping and the Human Genome U-133 Plus 2.0 array will be used for expression analysis. Blood samples for genetic analysis will be collected using standard phlebotomy techniques into Vacutainer^{TM} tubes containing special preservatives for subsequent isolation of RNA and DNA. Blood for RNA isolation will be collected at approximately the same time of day for each individual for all three collection time points to control for circadian rhythm effects on expression.

Two blood samples will be collected from every individual who consented to genetics analysis for genotyping (PAXgene^{TM} Blood DNA). Blood samples for expression analysis will be collected into PAXgene^{TM} Blood RNA Tubes. All of these samples will be sent to Expression Analysis for processing. Detailed procedures for the proper collection of blood for genotyping and expression analysis and shipping to Expression Analysis are provided in the study's Operations Manual. Blood must be shipped the same day that it was collected and only should be collected on Mondays through Wednesday to assure delivery to the laboratory during the work-week.

Gene expression and SNP profile data produced at Expression Analysis will be submitted to Information Management Consultants (IMC) for data warehousing and analysis.

13.6.31 Substance Use Report (SUR)

The SUR includes the subject's report of use of methamphetamine, marijuana, nicotine, alcohol, opiates, and cocaine use for each day of the week. The subject is asked to report any use during days since the last clinic visit. The day that the subject is reporting use is not scored until the subsequent visit as use may occur later in the day.

13.6.32 Timeline Follow-back

Detailed histories of methamphetamine use over the past 30 days prior to screening will be obtained using the timeline follow-back method. The timeline followback method was described and validated by Sobell et al.,[151] for reporting alcohol use. It has also been found to be a reliable method for assessing the history of psychoactive substance use in drug-abusing populations.[152]

13.6.33 Vital Signs

Vital signs to be assessed include oral temperature, sitting blood pressure, pulse rate, and respiratory rate.

14 REGULATORY AND REPORTING REQUIREMENTS

14.1 Form FDA 1572

The investigator at each study site will sign a Statement of Investigator (Form FDA 1572) prior to initiating this study.

14.2 IRB Approval

Prior to initiating the study, the investigator at each study site will obtain written IRB approval to conduct the study.   Should changes to the study protocol become necessary, protocol amendments will be submitted in writing to the IRB by the investigator for IRB approval prior to implementation. In addition, IRBs and NIDA will approve all advertising materials used for subject recruitment and any educational materials given to the subject. Annual reports and progress reports will be submitted to the IRB annually or at a frequency requested by the IRB.

14.3 Informed Consent

All potential subjects for the study will be given a current copy of the Informed Consent Forms to read and take home. A separate consent will be used for the genetics portion of the study. Subjects do not have to agree to participate in the genetics portion of the study to be eligible to participate in the study.

The investigator, sub-investigators, or study physician or designated staff at each site will explain all aspects of the study in lay language and answer all of the subject's questions regarding the study. If the subject desires to participate in the study, s/he will be asked to sign the Informed Consent(s). No study procedure will be performed prior to signing Informed Consent. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice. All study subjects will be given a copy of the signed Informed Consent(s).

14.4 Drug Accountability

Upon receipt, the investigator/pharmacist or a licensed designate is responsible for taking inventory of the investigational products. A record of this inventory must be kept and usage must be documented. Any unused or expired investigational products shall be returned to the Sponsor or Murty Pharmaceuticals after accountability at the site has been completed.

14.5 Outside Monitoring

Data and Safety Monitoring Board: Safety data will be reviewed by a Data and Safety Monitoring Board (DSMB) that will meet quarterly after enrollment begins. Additional meetings may be held on an *ad hoc* basis. The board will be blinded to subjects' actual study assignments, but may break the blind if safety concerns arise from the blinded data.

Medical Monitor: The NIDA medical monitor will be available for making recommendations to the investigator on the severity of any SAEs, the relatedness to the study medication, and for determining if an SAE should be reported to the FDA in a 7- or 15-day expedited report or an

annual report (**Appendix I**). The NIDA medical monitor will also be responsible for tracking and assessing trends in the SAEs reported.

Clinical Monitors: All investigators will allow representatives of the Sponsor to periodically monitor, at mutually convenient times during and after the study, all eCRFs and corresponding source documents for each subject. These monitoring visits provide the Sponsor with the opportunity to evaluate the progress of the study and to inform the Sponsor of potential problems at the study sites. The monitors will assure that submitted data are accurate and in agreement with source documentation; verify that investigational products are properly stored and accounted for, verify that subjects' consent for study participation has been properly obtained and documented, confirm that research subjects entered into the study meet inclusion and exclusion criteria, and assure that all essential documentation required by good clinical practices guidelines are appropriately filed.

Monitors will conduct a site initiation visit prior to the start of the study. At this visit, they will assure that proper study-related documentation exists, assist in training investigators and other site personnel in study procedures and compliance with good clinical practice guidelines and FDA regulations, confirm receipt of study supplies, and assure that acceptable facilities are available to conduct the study.

Routine monitoring visits by the Sponsor's representatives will be scheduled at appropriate intervals but more frequently at the beginning of the study. At these visits, the monitors will verify that study procedures are being conducted according to the protocol guidelines. At the end of the study they will advise on storage of study records and return of unused investigational products. All sites should anticipate visits by NIDA and the FDA.

14.6 Adverse Events Reporting

In accordance with FDA reporting requirements, all AEs occurring during the course of the clinical trial will be collected, documented, and reported by the principal investigator or sub-investigators according to the specific instructions detailed in this section of the protocol and Appendix I. The occurrence of AEs will be assessed at each study visit starting at the time that informed consent is signed through the end of follow-up at Week 16.

An AE is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial, whether or not the event is considered investigational product-related or clinically significant**.** For this study, AEs will include events reported by the subject, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant clinical laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. Stable chronic conditions, such as arthritis, which are present prior to clinical trial entry and do not worsen are not considered AEs. All AEs must be recorded on the AE Form. The AE Form is also used to record follow-up information for unresolved events reported on previous visits.

Each week, a study investigator must review the AE Form completed for the previous week for any events that were reported as continuing. All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed by study investigators

until satisfactory resolution. AEs should be reported up to 4 weeks following completion of, or termination from the intervention phase of the study.

14.7 Serious Adverse Events

Each adverse event or reaction will be classified by the study investigator as serious or non-serious. Based on the seriousness of the adverse event or reaction appropriate reporting procedures will be followed. The International Conference on Harmonization (ICH) Guideline for Industry: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH-E2A March 1995, as implemented by the U.S. Food and Drug Administration defines serious adverse event (SAE) or reaction as any untoward medical occurrence that at any dose:

- results in death;

- is life-threatening; *(NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.)*

- requires inpatient hospitalization or prolongation of existing hospitalization;

- results in persistent or significant disability/incapacity; or

- is a congenital anomaly/birth defect.

In addition, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug reaction, when based on appropriate medical judgment that may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the above definition. Subjects that become pregnant during study participation will be taken off study drug. However, subjects may continue to receive counseling for substance abuse or be referred for appropriate treatment. Subjects will be asked to sign a release of information form for study personnel to access medical records to obtain information regarding the outcome of the pregnancy.

An unexpected event is one that is not described with respect to nature, severity, or frequency in the current Investigator's Brochure or product package insert.

Reporting of AEs and SAEs is described in **Appendix I.** There can be serious consequences including ultimately, criminal and/or civil penalties for Sponsors who fail to comply with FDA regulations governing the reporting of SAEs to FDA. The study investigators in this study have the responsibility of promptly reporting all SAEs to NIDA in order that the NIDA, as the IND Sponsor, can comply with these regulations.

If a study subject withdraws from the study or if an investigator decides to discontinue the subject from the study because of a SAE, the subject must have appropriate follow-up medical monitoring. If the subject is hospitalized, medical monitoring will consist of not less than daily

evaluation by physical examination, vital signs, laboratory evaluations, and if applicable, ECG monitoring for significant treatment-emergent abnormalities. Monitoring will continue until the problem prompting hospitalization has resolved or stabilized with no further change expected or is discovered to be clearly unrelated to of investigational products or progresses to death.

15 ANALYTICAL PLAN

15.1 Statistical Hypotheses

Primary Outcome: It is hypothesized that bupropion, as compared to placebo, will increase the proportion of subjects who achieve abstinence (confirmed by at least two methamphetamine-negative urines and no methamphetamine-positive urines) during the last two weeks (Weeks 11 and 12) for non-daily users.

Secondary Outcomes: It is hypothesized that bupropion, compared to placebo, will be associated with an increase in the proportion of subjects who achieve abstinence (confirmed by at least two methamphetamine-negative urines and no methamphetamine-positive urines) each week during the last two weeks (Weeks 11 and 12) for subjects using methamphetamine 18 or less days during the 30 days prior to the start of signing consent. Secondly, it is also hypothesized that bupropion, as compared to placebo, will increase the proportion of subjects with 21 consecutive days of abstinence during which time all urine drug screens must be methamphetamine-negative (both with and without self report), increase the proportion of subjects who reduce their methamphetamine use as compared to baseline use, increase the weekly mean proportion of methamphetamine non-use days according to self-report alone, increase the proportion of methamphetamine negative urine samples, and improve treatment retention. It is hypothesized that bupropion will reduce the severity of methamphetamine dependence, craving, and comorbidity as assessed by the ASI-Lite, the BSCS, the CGI-S, the CGI-O, and the AISRS. It is further hypothesized that in the evaluable population (as defined in section 15.3), bupropion will be significantly more effective than placebo in those subjects who are medication-compliant compared to either the placebo group or subjects who were not medication compliant. This applies to the primary outcome and all secondary outcomes. Medication-compliant subjects are defined as individuals testing positive for bupropion and/or its major metabolite in at least 50% of urine samples (in aggregate) obtained during Weeks 1 through 10 and at least 66% of urine samples (in aggregate) obtained during Weeks 11 and 12.

15.2 OUTCOME MEASURES

The primary outcome measure, abstinence at the end of the 12-week investigational product dosing period confirmed by two weeks of methamphetamine negative urine specimens is considered a clinically significant improvement in methamphetamine use in this study population. In addition, the secondary outcome variables are intended to explore various aspects of the response to bupropion and to identify factors that may be associated with a treatment success. The primary outcome has been chosen because it is an objective measure of stopping methamphetamine use. Some of the secondary outcome variables add a measure of clinical relevance to the reduction of use by requiring either sustained abstinence (21 Days) or a predetermined, substantial overall reduction in use days (25% and by 50% of their baseline use).

15.2.1 Primary Outcome Measure

The primary efficacy outcome measure is a binary measurement of treatment success or failure, where a subject who successfully achieves two weeks of abstinence during the last two weeks of the investigational product dosing period (Weeks 11 and 12) is scored as a success. During the investigational product dosing period, each subject is asked to provide three urine samples per week. A successful outcome requires (1) all urine samples in the last two weeks are negative for methamphetamine (< 300 ng/mL quantitative result of negative qualitative result) and (2) a minimum of two urines samples collected and tested per each of the last two weeks during investigational product dosing. A subject who drops out before the last 2 weeks of the treatment period is scored as a failure in the analysis. A subject who stays on study to the beginning of Week 11 but who does not provide at least 2 urine samples per week during Weeks 11 and 12 is scored as a failure.

15.2.2 Secondary Outcomes Measures
Effect on methamphetamine and other drug use during Study Weeks 1-12 (except J)

A. The secondary efficacy outcome measure is also a binary measurement of treatment success or failure, where a subject who successfully achieves two weeks of abstinence during the last two weeks of the investigational product dosing period (Weeks 11 and 12) is scored as a success. The study population for this outcome measure is defined as those subjects with methamphetamine dependence who report using methamphetamine 18 or less days during the 30 days prior to signing consent.

B. Methamphetamine non-use weeks during study Weeks 1 through 12. Use weeks are defined as each 7-day period staring with the first day of investigational product administration. A non-use week is any week in which all of the urine drug screens for methamphetamine were negative (< 300 ng/mL). Conversely, a use week is any week in which at least one of the urine drug screens for methamphetamine was positive ($\geq$ 300 ng/mL). If no drug screening results are available, the data for that week is considered as missing.

C. Log_{10} weekly mean quantitative urine methamphetamine level.

D. The proportion of successful subjects with different patterns in the reduction in drug use examined as follows:

  1. The proportion of subjects with 21 consecutive days of abstinence, during which time all urine drug screens must be methamphetamine-negative (quantitative analysis <300ng/ml). Study days between methamphetamine negative urine specimens are considered to be methamphetamine-negative days. If more than three calendar days of urine specimens are missing, then this period is not considered to be abstinent.

  2. The proportion of subjects with 21 consecutive days of abstinence, during which time all urine drug screens must be methamphetamine-negative (quantitative <300 ng/mL) and there is no self-report of methamphetamine use (SUR). If one to seven calendar

days of urine specimens are missing, self-reported methamphetamine use will be used for evaluation. If no use is reported and there are no missing self-reports during this time, this period will be considered abstinent. If more than seven calendar days of urine specimens are missing or if any self-report is missing, then this period is not considered to be abstinent.

3. The proportion of subjects who decrease the overall proportion of negative methamphetamine use days by SUR by 25% or more of their self-reported use in the 14-day baseline period.

4. The proportion of subjects who decrease the overall proportion of negative methamphetamine use days by SUR by 50% or more of their self-reported use in the 14-day baseline period.

5. The proportion of subjects who decrease the mean of the $\log_{10}$-transformed methamphetamine quantitative urine concentration by 25% or more of their mean methamphetamine $\log_{10}$-transformed quantitative urine concentration in the 14-day baseline period.

6. The proportion of subjects who decrease the mean of the $\log_{10}$-transformed methamphetamine quantitative urine concentration to 50% or less of their mean methamphetamine $\log_{10}$-transformed quantitative urine concentration in the 14-day baseline period.

E. Weekly mean proportion of methamphetamine non-use days based on subjects self report of use (SUR).

F. The maximum number of consecutive methamphetamine non-use days by subjects self report of use (SUR).

G. The overall proportion of methamphetamine negative urines.

H. Weekly mean proportion of non-use days of other drug use, by other drug according to SUR.

I. Weekly proportion of negative urines results for other drug use, by drug.

J. The maximum number of calendar days of abstinence during Study Weeks 1-12, during which time all urine drug screens must be methamphetamine-negative (quantitative <300 ng/mL). Study days between urine specimens are considered to be methamphetamine-negative days. If more than three calendar days of urine specimens are missing, then this period is not considered to be abstinent.

K. The maximum number of calendar days of abstinence during Study Weeks 1-12, during which time all urine drug screens must be methamphetamine-negative (quantitative <300 ng/mL) and there is no self-report of methamphetamine use. If one to seven consecutive days of urine specimens are missing, evaluate self-reported methamphetamine use. If no

use is reported and there are no missing self-reports during this time, this period will be considered abstinent. If more than seven calendar days of urine specimens are missing or if any self-report is missing, then this period is not considered to be abstinent.

L   Methamphetamine non-use weeks during study Weeks 1 through 8.

Reduction in the severity of methamphetamine dependence, craving, and withdrawal

M.   Change in CGI-O scores since baseline

N.   Change in CGI-S scores since baseline

O.   Change in ASI-Lite scores since baseline

P.   Change in BSCS scores since baseline

Severity of Depression

Q.   Change in HAM-D scores since baseline

Severity of ADHD Symptoms

R.   Change in AISRS Scores since baseline

S.   The overall proportion of subjects who show a 30% improvement in the AISRS score from baseline to Week 4, 8, 12 and the last available AISRS.

T.   Change in the hyperactive/impulsive score of the AISRS (score of even numbered questions of AISRS) from baseline to Week 4, 8, 12 and the last available AISRS.

U.   Change in the inattentive score the AISRS (score of odd numbered questions of AISRS) from baseline to week 4, 8, 12 and the last available score.

HIV Risk Taking Behaviors

V.   Change in HRBS scores since baseline

Treatment Retention

W.   Time from start of investigational product administration until study completion or early termination

Safety of Bupropion

X.   AEs, clinical laboratory data, physical exams, and vital signs.

Prevention of Relapse to Methamphetamine Use After Completion of Treatment

Y.   Proportion of subjects who met the criteria for being abstinent according to the primary outcome measure during Weeks 11 and 12 who continue to be abstinent Weeks 13 through 16 as determined by self-report and confirmation by methamphetamine negative urine sample.

Z.   Proportion of subjects who met the criteria for being abstinent according to the primary outcome measure who continue to be abstinent Weeks 13 through 16 as determined by self-report alone.

The proportions will be calculated using the number of subjects who meet the criteria for continued abstinence as described above by the number who were abstinent during weeks 11 and 12 as defined by the primary outcome measure.

Plasma Levels of Bupropion and BDNF

Bupropion is primarily metabolized in human liver by CYP 2B6, an isoform that shows high interindividual variability in expression and catalysis which has been hypothesized to account for the variability in treatment response and toxicity associated with bupropion. Rapid clearance of bupropion via hydroxylation could account for treatment failure, likewise, slow clearance could account for toxicity. Recently, the 6B haplotype of CYP 2B6 has been shown to be a significant predictor of bupropion hydroxylation in human liver microsomal preparations, thus suggesting that individuals with this haplotype may be poorer responders to treatment. At steady state, the mean C_{max} following a 150-mg dose of bupropion every 12 hours is 136 ng/mL. Bupropion plasma levels will be examined for correlation to CYP 2B6 haplotypes and to treatment success.

Elevated plasma BDNF levels are associated with methamphetamine neurotoxicity, and are known to decline during abstinence from methamphetamine. Since bupropion also has an independent effect on BDNF, plasma samples will be tested for bupropion level at the same timepoints as plasma for BDNF is obtained. This will allow the evaluation of whether BDNF is affected differently in subjects receiving bupropion compared to those receiving placebo, and whether this effect influences methamphetamine use or abstinence. In addition, it is also hypothesized that methamphetamine users who have the BDNF 66Val homozygote will be more likely to benefit from bupropion than those who do not.

15.2.3 Additional Secondary Outcome Measures and Analyses

The following additional analyses will be conducted:

i. A novel, non-binary method for evaluating success and failure (McCann and Li, submitted to CNS Neuroscience & Therapeutics) will be used to test the hypothesis that bupropion, as compared to placebo, will increase abstinence from methamphetamine that lasts for multiple weeks and through the end of the 12-week treatment phase. A subject will be regarded as abstinent from methamphetamine during a given week if at least two methamphetamine-negative urines and no methamphetamine-positive urines are provided, and a subject who achieves abstinence during Weeks 11 and 12 will be regarded as a success. A subject who drops out of

the study, provides one or more methamphetamine-positive urines during Weeks 11 and 12, or provides less than two urines per week during Weeks 11 and 12 will be regarded as a failure. For successful subjects, urine results from all weeks prior to Week 11 will be evaluated and, again, they will be regarded as abstinent from methamphetamine during those weeks with at least two methamphetamine-negative urines and no methamphetamine-positive urines. Their degree of success will then be calculated by determining the "Number of Beyond-Threshold Weeks of Success" (NOBWOS), with the threshold set at 1 week of end-of-study abstinence. For example, a subject achieving 8 weeks of end-of-study abstinence (abstinence on weeks 5 through 12) will be assigned a NOBWOS value of 7. Similarly, a subject who achieves the minimum level of success (abstinence on weeks 11 and 12) will be assigned a NOBWOS value of 1. Treatment failures will be assigned a NOBWOS value of 0. NOBWOS values will then be compared between groups using a two-sided Van der Waerden two-sample test.

ii. The relationship between self-reported baseline level of methamphetamine use (number of days reported use during the 30 days immediately prior to screening) and the percentage of urines positive for bupropion and/or its major metabolite throughout the 12-week treatment period for all subjects randomized to the bupropion group will be evaluated using a logistic regression analysis. The percentage of urines testing positive for bupropion and/or its metabolite will serve as the dependent variable and the number of days reported use prior to screening will serve as the independent variable.

iii. The relationship between the percentage of methamphetamine-positive urines during screening and the percentage of urines positive for bupropion and/or its major metabolite throughout the 12-week treatment period for all subjects randomized to the bupropion group will be evaluated using a logistic regression analysis. The percentage of urines testing positive for bupropion and/or its metabolite will serve as the dependent variable and the percentage of urines testing positive for methamphetamine during screening will serve as the independent variable.

15.3 "Intention-to-Treat," "Evaluable," and "Expanded Evaluable" Subject Populations

The ITT population is defined as subjects who were randomized and received at least 1 dose of investigational product. The evaluable population is defined as the subjects who are eligible to participate in the study in accordance with the inclusion and exclusion criteria and who received at least 4-weeks of investigational product, and provided at least 2 urine samples per week that were analyzed for methamphetamine during the last two weeks of the study (Weeks 11 and 12). The expanded evaluable population is defined as the subjects who are eligible to participate in the study and who received at least 4-weeks of investigational product, and provided at least 2 urine samples per week that were analyzed for methamphetamine during their last 2 weeks of participation in the trial (dropouts allowed).

15.4 Analysis Plan

15.4.1 Efficacy Assessments
Each of the primary and secondary efficacy outcome measures listed in sections 15.2.1 and 15.2.2 will be analyzed for the ITT population. The primary outcome measure also will be analyzed and for the "evaluable" and "expanded evaluable" populations. Each of these analyses will be conducted in two ways: 1) comparing the bupropion and placebo groups independent of

compliance data and 2) comparing the medication-compliant subjects in the bupropion group with both the placebo group and the subjects who were not medication-compliant in the bupropion group. Medication-compliant subjects are defined as individuals testing positive for bupropion or its major metabolite in at least 50% of urine samples (in aggregate) obtained during weeks 1 through 10 and at least 66% of urine samples (in aggregate) obtained during weeks 11 and 12. Finally, the analyses described in section 15.2.3 will be conducted for the ITT population.

While there is every intent to be complete in describing the analyses to be performed, it is not possible to anticipate every contingency and some adjustments may be required to meet constraints posed by the structure of the data.

Statistical tests will be two-sided at a 5% Type I error rate. Confidence intervals will be two-sided with a 95% confidence coefficient.

Primary Efficacy Outcome Measure

The primary efficacy outcome measure is a binary measurement of treatment success or failure, where a subject who successfully achieves two weeks of abstinence during the last two weeks of the investigational product dosing period (Weeks 11 and 12) is scored as a success. During the trial, each participant is asked to provide three urine samples per week. A successful outcome requires (1) all urine samples in the last two weeks are negative for methamphetamine (< 300 ng/mL quantitative result of negative qualitative result) and (2) a minimum of two urines samples collected and tested per each of the last two weeks during investigational product dosing. A subject who drops out before the last 2 weeks of the treatment period is scored as a failure in the analysis. A subject who stays on study to the beginning of Week 11 but who does not provide at least 2 urine samples per week during Weeks 11 and 12 is scored as a failure. The proportion of subjects achieving a successful outcome will be compared between groups using a Chi-square test or Fisher exact test, as appropriate based on the estimated expectation of the contingency table's cell sizes computed from the table's row and column totals.

As a secondary analysis, the individual effects, if any, of age, race, methamphetamine use (18 or less *versus* 19-29 days out of 30 days prior to signing consent), clinical site, diagnosis of adult ADHD, severity of depression (HAM-D score ≤ 12 *versus* >12), bupropion blood levels, and usual route of methamphetamine use (oral/nasal inhalation versus intravenous/smoked) on the primary study group effects will be determined where numbers permit.

Secondary Efficacy Outcomes

Unless the primary response analysis implies the need for a more elaborate model, between group comparisons of the secondary outcomes will be performed as follows:

Outcome Measure (from above)	Statistical Analysis Method
A, B, C, E, H, I, J, M, N, O, P, Q, R, T, U, V	GEE
D, S, Y, Z	Chi-square or Fisher's Exact tests
F, G, J, K	t-test or appropriate nonparametric test

15.4.2 Safety Data

The severity and frequency of adverse events, and laboratory data, physical exams, and vital signs, will be reported in tabular form. Adverse events will be coded using Medical Dictionary of Regulatory Activities (MedDRA) preferred terms and grouped by system, organ, and class (SOC). The frequencies of adverse events by type will be compared between study arms using Chi-square analyses; however, this analysis will be considered descriptive not inferential.

15.4.3 Descriptive Statistics

Summaries of the characteristics of the subject population in both groups at baseline will be prepared for both the ITT and evaluable subjects. A summary will be prepared to show dropouts/retention over time in each study group and for major subgroups. The number of missing observations will be compared between groups and for major subgroups. Weekly treatment retention will be presented. Investigational product compliance will be presented by investigational product reconciliation, and subject report of use.

15.4.4 Genetics Analyses

Genotyping and Association Analysis. Association analysis for either single or multiple SNPs (as a block) from GeneChip® Mapping 500K Array will be conducted by using the ALLELLE, CASECONTROL and HAPLOTYPE procedures of SAS/Genetics. The ALLELE procedure of the SAS/Genetics package can calculate descriptive statistics such as the frequency and variance of alleles and genotypes, as well as estimate measures of marker informativeness, test whether genotype frequencies are consistent with HWE (Hardy-Weinberg Equilibrium), and support three methods for calculation of the degree and significance of linkage disequilibrium (LD). Another useful procedure for the case-control design (as used in the current bupropion trial study) is CASECONTROL, which compares allele and genotype frequencies in bupropion-treated and placebo-treated groups using three types of Chi-square tests (i.e., genotype and allele case-control tests and linear trend test) and options for controlling correlation of allele frequencies among members of the same subpopulation. Similarly, we plan to test association using haplotype information from multiple SNPs within a candidate gene/region for the case-control data. The program to be used for this purpose is the HAPLOTYPE procedure of SAS/Genetics, which uses case-control data to calculate test statistics for the hypothesis of no association between alleles comparing the haplotypes and disease or invention group status. In addition, for the bupropion group, we also plan to examine the association between genetic markers and blood bupropion levels and safety outcomes such as frequency of severity of commonly reported AEs (e.g., insomnia). Furthermore, we plan to analyze interactions between significant genes or SNPs and environmental factors using a generalized multifactor dimensionality reduction method developed by Li and his colleagues at the University of Virginia.[153]

Microarray Data Analysis. All microarray data generated from Affymetric Human Genome U-133 Plus 2.0 array will be subjected to a series of steps for normalization and filtering, which includes log-transformation, normalization within a slide, scaling among slides, and data filtering. To reduce individual variation, we will use expression profile of each subject prior to administration of investigational products as the baseline. All expression profiles of that

individual at other time points will be compared with the baseline. After appropriate normalization, various statistical approaches will be applied to select genes differentially expressed in the bupropion and placebo groups. Computer programs written in SAS, Fortran, or MatLab for the traditional *t*-test, the SAM method (a modified *t*-test), variance analysis under a mixed model, and a mixture-model approach have been implemented in our laboratory and are ready for handling the data generated from this project. Given that each method has its own features, and there is no clear standard or consensus as to which method is preferable, we plan to use all of these methods to analyze each dataset. Only those genes that can be identified repeatedly by different methods will be selected for further characterization.

After differentially expressed genes are identified, we plan to use various clustering techniques over different experimental groups to analyze the expression patterns. Expression profiles will be grouped by: a) hierarchical cluster analysis using CLUSTER and TREEVIEW (http://rana.stanford.edu/software/), b) quality clustering (http://www.genome.org/cgi); and c) self-organizing maps (http://www-genome.wi.mit.edu/). The outputs from each program will be compared with respect to the clusters formed. These data will be used to determine relationships between the clusters, disease status, and intervention group and response. These methods have been routinely used in our microarray data analysis.[68,154-156]

15.5 Sample Size calculation

Sample size estimates were performed using the last two week's abstinence rates from a prior Phase 2 trial examining the efficacy of bupropion for the treatment of methamphetamine dependence. The proportion of successful participants was 0.238 and 0.057 for the bupropion and placebo arms, respectively. The proportion of participants achieving a successful outcome was compared between arms using a two-tailed Fisher's exact test (p = 0.01). For a power of 95% and a Type I error rate of 5%, a sample size of 100 per group (total of 200 subjects) is required.

15.6 Control of Bias

Adaptive randomization will be used to balance study intervention groups based on clinical site, methamphetamine use (18 or less versus 19-29 days out of 30 days prior to signing consent),, severity of depression symptoms (HAM-D score ≤ 12 *versus* >12], and presence of adult ADHD.

15.7 *Post Hoc* Analyses

Data will be collected in this study for scientific use and not as primary or secondary outcome measures. These data are being collected to build a database that will help characterize the study population. Additional *post hoc* analysis may be performed to evaluate other confounding factors on outcomes such as depression, diagnosis of adult ADHD, or patterns of methamphetamine use at baseline and after completion of interventions.

16 DATA MANAGEMENT AND ELECTRONIC CASE REPORT FORMS (ECRF)

General Training. Good clinical practices (GCP) and study specific training will be held for all research staff. This training will include an overview of GCPs, site's training manuals, and the

study operations manual. Staff members will also receive copies of the study protocol, eCRFs, and operations manual. These materials will be reviewed with the full staff.

Operations Manual. An operations manual will be provided for this study that incorporates procedures from this protocol with those procedures necessary for the day-to-day conduct of the trial. The operations manual will be used to train study staff, to provide reference for study procedures, and to guide quality assurance activities.

16.1 Data Collection

Data will be collected at the study sites on source documents, which will be entered at the site into eCRFs. The eCRFs will be supplied by the Data Management Center. eCRFs are to be completed on an ongoing basis during the study. The medical chart and the source documents are the source of verification of data. eCRFs should be completed according to the instructions in the study operations manual. The principal investigator is responsible for maintaining accurate, complete and up-to-date records for each subject. The principal investigator is also responsible for maintaining any source documentation related to the study, including any films, tracings, computer discs or tapes.

16.2 Data Editing and Control

Data entered into the eCRFs by the sites will be reviewed by KAI. If incomplete or inaccurate data are found a data clarification request will be forwarded to the sites for a response. Sites will resolve data inconsistencies and errors. All corrections and changes to the data will be reviewed by KAI. NIDA/DPMC and the participating sites will receive reports at least monthly regarding the quality and quantity of data submitted to Data Management Center.

Participating investigators agree to routine data audits by the Sponsor's designated staff, audits by the staff of the Data Management Center and by NIDA's programmatic staff. Monitors will routinely visit each site to assure that data entered on the appropriate eCRFs are in agreement with source documents. They will also verify that of investigational products have been properly stored and accounted for, subject informed consent for study participation has been obtained and documented, all essential documents required by GCP regulations are on file, and sites are conducting the study according to the research protocol. Any inconsistencies will be resolved, and any changes to the data forms will be made using the established procedures specified in the study operations manual.

16.3 Data Entry, Processing, and Analyses

Data will be collected at the study sites on source documents, which will be entered into eCRFs. When the study is completed and all data have been entered into the clinical database and the database has been checked by Quality Assurance and is locked, statistical analysis of the data will be performed by the Data Management Center's statisticians in accordance with the analytical plan section of this protocol. Periodically, during the investigation, data sets will be submitted to the NIDA DTR&D central data repository according to procedures specified in the study operations manual.

16.4 Study Documentation and Records Retention

Study documentation includes all data correction forms, workbooks, source documents, monitoring logs and appointment schedules, Sponsor correspondence and regulatory documents (e.g., signed protocol and amendments, Ethics or Institutional Review Committee correspondence and approved consent form and signed subject consent forms, Statement of Investigator form, and clinical supplies receipt and distribution records).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study. Accordingly, source documents include, but are not limited to, laboratory reports, ECG tracings, X-rays, radiologist reports, patient diaries, biopsy reports, ultrasound photographs, patient progress notes, hospital charts or pharmacy records and any other similar reports or records of any procedure performed in accordance with the protocol.

Whenever possible, the original recording of an observation should be retained as the source document; however, a photocopy is acceptable provided that it is a clear, legible, and an exact duplication of the original document.

Government agency regulations and directives require that the investigator must retain all study documentation pertaining to the conduct of a clinical trial. These documents must be kept for a minimum of two years after discontinuation of the IND or 2 years after the approval of the NDA.

16.5 CONFIDENTIALITY

16.5.1 Confidentiality of Data
Particular attention is drawn to the regulations promulgated by the Food and Drug Administration (FDA) under the Freedom of Information Act providing, in part, that proprietary information furnished to clinical investigators and Institutional Review Boards will be kept confidential by the FDA only if maintained in confidence by the clinical investigator and Institutional Review Board (IRB).

By signing this protocol the investigator affirms to NIDA that information furnished to the investigator by NIDA will be maintained in confidence and such information will be divulged to the IRB or similar or expert committee; affiliated institution; and employees only under an appropriate understanding of confidentiality with such board or committee, affiliated institution and employees.

16.5.2 Confidentiality of Patient Records
To maintain subject confidentiality, all laboratory specimens, eCRFs, reports and other records will be identified by a subject identification code that includes the site number, subject number, and subject alpha code. Research and clinical records will be stored in a locked cabinet. Only research staff and NIDA program officials will have access to the records. Subject information will not be released without written permission, except as necessary for monitoring by the FDA, the NIDA monitoring contractor, or NIDA. Upon approval of the study by an IRB, an application will be filed with NIDA for a certificate of confidentiality.

By signing the protocol the investigator agrees that within local regulatory restrictions and ethical considerations, NIDA or any regulatory agency may consult and/or copy study documents in order to verify eCRF data.
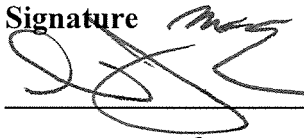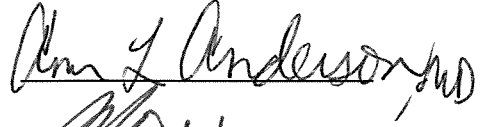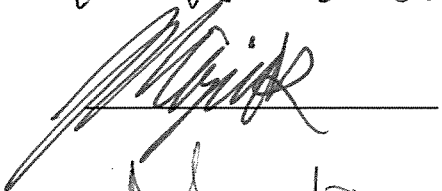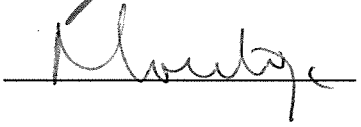
The procedure for applying for a certificate of confidentiality is provided in Appendix II.

17 PUBLICATIONS OF THE STUDY RESULTS

NIDA and the investigative group agree that each participating site's database will be made available to individual investigators to encourage other publications, either by a group or by an individual investigator provided that: manuscripts based on the use of bupropion for the treatment for methamphetamine dependence may not be submitted for publication until the main findings of the study have been published and this study has been accepted by the FDA for filing to the IND or NDA.

18 SIGNATURES

NIDA REPRESENTATIVES

Typed Name	Signature	Date
David McCann, Ph.D. Protocol Chairman		April 18, 2011
Ann Anderson, M.D. NIDA Principal Investigator		18 Apr 2011
Jurij Mojsiak, M.S. NIDA Project Director		18 Apr 2011
Ivan Montoya, M.D. NIDA Medical Monitor		18 Apr 2011

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APPENDIX I: *Instructions for Evaluating and Reporting Adverse Events and Serious Adverse Events*

A. GENERAL INSTRUCTIONS

1. Adverse Events (AEs) will be assessed at each visit and reviewed weekly by a study physician.

2. Record AEs as soon as the informed consent process is completed. AEs reported before investigational products are administered will be reported separately from those that occur after the start of investigational product administration.

3. Report the severity of the event following the guidance in section B below.

4. Report the relatedness of the event to the investigational product administration according to the guidance in section C.

B. DEFINITIONS – SEVERITY OF EVENTS

Mild: Awareness of symptom, but easily tolerated.

Moderate: Discomfort enough to cause interference with usual activity.

Severe: Incapacitating with inability to work or do usual activity.

C. DEFINITIONS – RELATEDNESS OF EVENTS

The study physician is responsible for defining, in his/her best judgment, the relationship of the AE/SAE to the study drug/placebo. The degree of certainty for which the AE/SAE is attributed to the study drug or alternative causes (e.g. natural history of the underlying disease, concomitant therapies, etc.) should be determined by how well the experience can be understood in terms of one or more of the following:

*   *Exposure:* Is there evidence that the subject was actually exposed to the drug/placebo?

*   *Timing of the study drug/placebo:* Did the AE/SAE follow in a reasonable temporal sequence from administration of the drug test?

*   *Consistency with study drug profile:* Known pharmacology and toxicology of the study drug in animals and man; reaction of similar nature having been previously described with the study drug.

*   *Alternative explanations* for the adverse event such as concomitant medications, concurrent illness, non-medicinal therapies, diagnostic tests, procedures or other confounding findings.

*   *Response to discontinuation* of the study drug/placebo.

Terms and definitions to be used in assessing the investigational product relationship to the AE/SAE are:

- *Unknown:*
  Use this category only if the cause of the AE/SAE is not possible to determine

- *Definitely Not Related:*
  The subject did not receive the test drug, the temporal sequence of the AE/SAE onset relative to administration of the test drug is not reasonable, or there is another obvious cause of the AE/SAE.

- *Remotely Related:*
  There is evidence of exposure to the test drug or there is another more likely cause of the AE/SAE.

- *Possibly Related:*
  There is evidence of exposure to the test drug, the temporal sequence of the AE/SAE onset relative to administration of the test drug is reasonable, but the AE/SAE could have been due to another equally likely cause.

- *Probably Related:*
  There is evidence of exposure to the test drug, the temporal sequence of the AE/SAE onset relative to administration of the test drug is reasonable, and the AE/SAE is more likely explained by the test drug than by any other cause.

- *Definitely Related:*
  There is evidence of exposure to the test drug, the temporal sequence of the AE/SAE onset relative to administration of the test drug is reasonable, the AE/SAE is more likely explained by the test drug than by any other cause, and the AE/SAE shows a pattern consistent with previous knowledge of the test drug or test drug class.

D. SPECIFIC INSTRUCTIONS – LABORATORY/ECG ADVERSE EVENT

A laboratory or ECG AE is any clinically significant worsening in a test variable that occurs during the course of the study, whether or not considered to be investigational product related. For each such change, provide the information requested on date of test, severity, likelihood of a relationship to investigational product, change in investigational product dosage due to the AE, and treatment required.

All laboratory AEs should be specified as an increased or decreased test result (e.g. "increased glucose", "decreased potassium") or as a term that implies an abnormality (e.g., hypercalcemia, azotemia, hypokalemia, or bradycardia). Any abnormal laboratory value that is considered not clinically significant will be recorded as such on the clinical laboratory report along with a comment providing justification for that determination.

E. SERIOUS ADVERSE EVENT AND UNEXPECTED ADVERSE EVENT REPORTING

24 hour Reporting Requirements

Any SAEs due to any cause, that occur during the course of this investigation, whether or not related to the investigational agent, must be reported within 24-hours by email, telephone or entered into the Serious Adverse Event Tracking and Reporting System (SAETRS) to: the Study Medical Monitor and the NIDA Project Director as follows:

NIDA Medical Monitor: Roberta Kahn, M.D. 301/443-2281

NIDA Project Director: Jurij Mojsiak, M.S. 301/443-9804

The following information must be provided with the initial report of an SAE or unexpected AE:

- Name of person reporting the SAE/unexpected AE
- Subject's I.D. number
- Name of the principal investigator and institution
- Date the subject signed informed consent
- Date of first dose of study medication
- Description of the SAE/unexpected AE
- Date and time of Onset
- Date/time of administration of last dose of study agent/placebo prior to the SAE/unexpected AE
- Severity of the SAE/unexpected AE
- Investigator's assessment of the relationship of the SAE/unexpected AE to study drug (related, possibly related, probably related, unlikely related, not related)
- Any action taken with the study drug, alteration to protocol defined schedule, diagnostics, and treatments secondary to the SAE/unexpected AE.

3-day Supporting Documentation Requirements

SAETRS will be used for recording all SAEs in this trial. Documentation for all SAEs/unexpected AEs must be received by the NIDA Medical Monitor and the NIDA Study Director within 3 days of reporting the event. Required documents that must be submitted include the following:

- SAE entry in SAETRS

Additional documentation may include:

- Adverse Events CRF pages
- Copies of source documents pertinent to the event (lab reports, ECG tracings, medical chart notes, etc.)

- Any other relevant information necessary to facilitate the investigator's judgment regarding the SAE's relatedness to the severity OR by request of the Medical Monitor/Alternate

These documents may be submitted by facsimile, as email attachments, or via overnight courier.

Follow-Up of All Adverse Events/Serious Adverse Events

All adverse medical events must be followed until they are resolved, or until all attempts to determine the resolution of the AE/SAE are exhausted. This may require an extended hospitalization period or a change in status from outpatient to inpatient. All treatments, outcomes and information regarding whether or not the subject was referred to their Primary Care Provider for additional follow-up must be recorded in the source document. All serious and unexpected adverse events occurring 30 days after administration of the last dose of study drug/placebo must be reported. All follow-up week 16 AEs will be recorded and followed to resolution only if they are serious, or if the study physician assesses them to be clinically significant.

The site investigator is required to provide the Medical Monitor and the NIDA Study Director with all relevant follow-up information necessary to facilitate a thorough understanding of the event and judgment regarding the relationship to the study drug/placebo.

Reporting to the FDA

The IND sponsor is required to report SAEs to the FDA:

- in 7 days if the SAE is unexpected (or, if expected, unusually serious or rarely seen), life-threatening or lethal, and at least possibly related to the investigational product, with a follow-up written report in 8 days;

- in 15 days if the SAE is unexpected (or, if expected, unusually serious or rarely seen), but not immediately life-threatening; and

- in an annual report in all other cases.

APPENDIX II: Procedure for Applying for a Certificate of Confidentiality

The only people who will know the identity of the subjects are members of the research team and, if appropriate, the physicians and nurses. No information about the subjects, or provided by the subjects during the research, will be disclosed to others without the subjects' written permission, except:

- if necessary to protect subjects' rights or welfare

When the results of the research are published or discussed in conferences, no information will be included that would reveal subjects' identity. Authorized representatives of the FDA and NIDA study monitors may need to review records of individual subjects. As a result, they may know subjects' names, but they are bound by rules of confidentiality not to reveal their identity to others. The results of this study including laboratory results and clinical information collected during this study will be submitted to the FDA and may be used for research purposes. The results of this study may be published but will not personally identify any subjects. All records will be kept in locked storage locations that will be accessible only to authorized study personnel.

NIDA will apply for a Certificate of Confidentiality for all participating sites.

This Certificate of Confidentiality helps researchers protect the privacy of subjects in health research projects against compulsory legal demands (e.g., court orders and subpoenas) that seek the names or other identifying characteristics of research subjects. The certificate was developed to protect against the involuntary release of personally identified research information of a sensitive nature sought through any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. This authority was granted under the Comprehensive Drug Abuse Prevention and Control Act of 1970, Public Law No. 91-513, Section 3(a).

This certificate is necessary for investigators to avoid being required to involuntarily disclose personally identifiable research information about individual study subjects. Under this statute:

"The Secretary [of the Department of Health and Human Services] may authorize persons engaged in biomedical, behavioral, clinical, or other research (including research on mental health, and on the use and effect of alcohol and other psychoactive drugs) to protect the privacy of individuals who are the subject of such research by withholding from all persons not connected with the conduct of such research the names or other identifying characteristics of such individuals. Persons so authorized to protect the privacy of such individuals may not be compelled in any Federal, State, or local civil, criminal, administrative, legislative, or other proceedings to identify such individuals" (Public Health Service Act 301 (d), 42 U. S. C. 241 (d), as amended by Public Law No. 100-607, Section 163 (November 4, 1988))."

Accordingly, this special privacy protection can be granted only to research (i.e., a systematic investigation, designed to develop or contribute to generalizable knowledge). It is granted only when the research is of a sensitive nature where the protection is judged necessary to achieve the research objectives.

The study subjects should be informed that a Certificate is in effect, and be given a fair and clear explanation of the protection it affords, including the limitations and exceptions. This information will be included in the informed consent. Please see below some suggested wording:

"We have received a Certificate of Confidentiality from the National Institute on Drug Abuse, which will help us protect your privacy. The Certificate protects against the involuntary release of information about your participation in this study. The researchers involved in this project cannot be forced to disclose your identity or your participation in this study in any legal proceedings at the federal, state, or local level, regardless of whether they are criminal, administrative, or legislative proceedings. However, you or the researcher may choose to voluntarily disclose the protected information under certain circumstances. For example, if you or your guardian requests disclosure of your participation, the researchers will provide research data. The Certificate does not protect against that voluntary disclosure.

Furthermore, federal agencies may review our records under limited circumstances, such as a DHHS request for information for an audit or program evaluation or a Food and Drug Administration request under the Food, Drug and Cosmetics Act."

<div align="center">or</div>

"A Certificate of Confidentiality has been obtained from the Federal Government for this study to help insure your privacy. This Certificate means that the researchers cannot be forced to tell people who are not connected with the study, including courts, about your participation, without your written consent. If we see [learn] something that would immediately endanger you, your child, or others, we may discuss it with you, if possible, or seek help."

Study subjects will be notified that a Certificate has expired if they are recruited to the study after the expiration date of the Certificate and an extension of the Certificate's coverage has not been granted.

If the research scope of a project covered by a Certificate should change substantially, the PI will request an amendment to the Certificate; however, the NIDA Certificate Coordinator may require a new Certificate depending on the extent of the change in scope. An extension of coverage must be requested if the research extends beyond the expiration date of the original Certificate, as research information collected after the expiration of a Certificate is not protected from compelled release.

A Certificate of Confidentiality is a legal defense against a subpoena or court order, and is to be used by the researcher to resist disclosure. The researcher should seek legal counsel from his or her institution if legal action is brought to release personally identifying information protected by a certificate. The Office of General Counsel for DHHS is willing to discuss the regulations with the researcher's attorney.