Investigator's Brochure

Product: (+/-)3,4-methylenedioxymethamphetamine (Laneo™ MDMA)
Edition: Fourth

Table of contents

LIST OF TABLES **4**
LIST OF FIGURES **5**
LIST OF ABBREVIATIONS **6**
1.0 SUMMARY **9**
2.0 INTRODUCTION **10**
3.0 PHYSICAL, CHEMICAL AND PHARMACEUTICAL PROPERTIES OF FORMULATION **13**
4.0 NONCLINICAL STUDIES **15**
4.1 NONCLINICAL PHARMACOLOGY 15
4.1.1. Primary Pharmacodynamics 18
4.1.2 Secondary Pharmacodynamics 19
4.1.3 Safety Pharmacology 20
4.1.3.1 Central Nervous System 20
4.1.3.2 Cardiovascular system 23
4.1.3.3. Respiratory System 23
4.1.3.4 Hepatic System 23
4.1.3.5. Renal System 24
4.1.4 Pharmacodynamic Drug Interactions 24
4.2 PHARMACOKINETICS AND PRODUCT METABOLISM IN ANIMALS 25
4.2.1 Absorption 27
4.2.2 Distribution 27
4.2.3 Metabolism 28
4.2.4 Elimination 29
4.3 TOXICOLOGY 29
4.3.1 Single Dose Toxicology Studies 29
4.3.2 Repeat-dose toxicology studies 30
4.3.3 Genotoxicity 33
4.3.4 Reproductive and Developmental Toxicity 33
4.3.5 Other Toxicity studies 34
5.0 EFFECTS IN HUMANS IN CLINICAL SETTINGS **36**
5.1. PHASE 1 STUDIES 40
5.2. PHASE 2 STUDIES 43
5.3. PHASE 3 STUDY 47
5.4 PHARMACOLOGY IN HUMANS 50
5.4.1 Pharmacokinetics and Product Metabolism in Humans 50
5.4.1.1 Pharmacokinetic Drug Interactions 53
5.4.2 Pharmacodynamics 54
5.4.3 Effect on the Central Nervous System 56
5.4.4 Behavioral Effects 57
5.5 EFFICACY OF MDMA 60
5.5.1 MAPP 1 – Pivotal phase 3 clinical trial 61
5.5.2 MAPP 2 – Confirmatory phase 3 clinical trial 68

5.5.3 MPLONG – Long term follow-up study 69
5.5.4 PTSD and mild alcohol or cannabis use disorder 70
5.5.5 PTSD and eating disorder 72
5.6 SAFETY OF MDMA 73
5.7 VITAL SIGNS 75
6.0 SUMMARY OF DATA **78**
6.1 PHARMACOLOGY 79
6.2 TOXICOLOGY 80
6.3 SAFETY IN HUMANS 81
6.3.1 ADVERSE EVENTS 81
6.3.2 SUICIDAL IDEATION AND BEHAVIOR 84
6.3.3 IMMUNOLOGICAL EFFECTS 85
6.3.4 HEPATIC EFFECTS 85
6.4 RISK ASSESSMENT 86
7.0 CONCLUSION **87**
8.0 BIBLIOGRAPHY **89**

List of Tables

TABLE 1: SUMMARY OF NONCLINICAL TOXICOLOGY STUDIES AVAILABLE IN THE LITERATURE[15] 16
TABLE 2: PHARMACOKINETIC CONSTANTS FOR PLASMA MDMA AFTER VARIOUS ROUTES OF ADMINISTRATION TO HUMANS OR ANIMALS.[15] 26
TABLE 3: SUMMARY OF MAPS SPONSORED TRAILS WITH MDMA 37
TABLE 4 PLASMA PK PARAMETERS OF MDMA FOLLOWING A SINGLE DOSE 120MG ORAL DOSE OF MDMA HCL UNDER FASTING OR FED CONDITIONS (STUDY MPKF) REF: 73,74 40
TABLE 5: TREATMENT EMERGENT ADVERSE EVENTS (PHASE 2 MDMA-ASSISTED PSYCHOTHERAPY) 45
TABLE 6: NUMBER OF PARTICIPANTS REPORTING TREATMENT-EMERGENT PSYCHIATRIC ADVERSE EVENTS IN THE PHASE 2 CLINICAL TRIAL WITH AUTISTIC ADULTS [57] 47
TABLE 7: DEMOGRAPHICS AND BASELINE CHARACTERISTICS ACROSS MAPP1 AND MAPP2 - PHASE 3 CLINICAL TRIALS[9,14] 49
TABLE 8 CHANGE IN SELF-EXPERIENCE SCORES BY TREATMENT GROUP- INTERACTION TERMS AND MAIN EFFECTS 66
TABLE 9 CHANGE IN CAPS-5 TOTAL SEVERITY CHANGE SCORES BY TREATMENT GROUP–INTERACTION TERMS AND MAIN EFFECTS. 66
TABLE 10 CAPS-5 PTSD TOTAL SEVERITY SCORES BY TREATMENT GROUP AND BASELINE SELF-EXPERIENCE LEVELS. 67
TABLE 11: PARTICIPANTS IN PHASE 3 CLINICAL TRIAL (MAPP1) WITH TREATMENT-EMERGENT SAES AND AESIS 74
TABLE 12: VITAL SIGNS OF VOLUNTEERS FROM MAPS SPONSORED TRIALS[15,57] 76
TABLE 13 TEAES OF SUICIDAL IDEATION, INTENTIONAL SELF-INJURY, SUICIDAL BEHAVIOR, SUICIDE ATTEMPT, AND SELF-INJURIOUS BEAHVIOR (MAPP PHASE 3 POOL) 85

List of Figures

FIGURE 1: CHEMICAL STRUCTURE OF LANEO(TM) MDMA HCL 14
FIGURE 2: METABOLISM OF MDMA IN HUMAN (RED) COMPARED TO METABOLISM IN RATS (IN BLUE). 28
FIGURE 3 ACUTE SUBJECTIVE EFFECTS OF 125 MG MDMA, 125 MG S-MDMA, 125 MG R-MDMA, AND 250 MG R-MDMA ON THE VISUAL ANALOG SCALE (VAS) 42
FIGURE 4 ACUTE AUTONOMIC EFFECTS OF R-MDMA, S-MDMA, MDMA AND PLACEBO IN HEALTHY HUMAN VOLUNTEERS 43
FIGURE 5 PLASMA CONCENTRATIONS OF OXYTOCIN, CORTISOL, AND PROLACTIN WITH MDMA, S-MDMA 43
FIGURE 6 MAPP1 AND MAPP2 CLINICAL DESIGN (PIVOTAL PHASE 3 STUDY DESIGN SPONSORED BY MAPS PBC/LYKOS THERAPEUTICS) 61
FIGURE 7: MEASURES OF MDMA EFFICACY IN THE MDMA-ASSISTED THERAPY GROUP AND THE PLACEBO GROUP.[14] 63
FIGURE 8: TREATMENT RESPONSE AND REMISSION FOR MDMA AND PLACEBO GROUPS AS A PERCENTAGE OF TOTAL PARTICIPANTS RANDOMIZED TO EACH ARM (MDMA, N = 46; PLACEBO, N = 44). 65
FIGURE 9: MEASURES OF EFFICACY IN THE MDMA-ASSISTED THERAPY AND PLACEBO WITH THERAPY GROUPS (MAPP2) 68
FIGURE 10: TREATMENT RESPONSE AND REMISSION IN MDMA-AT (N=53) AND PLACEBO WITH THERAPY (N=50) GROUPS (MAPP2).. 69
FIGURE 11 MPLONG CHANGE FROM PARENT STUDY BASELINE IN CAPS-5 TOTAL SEVERITY SCORE (EFFECTIVENESS SUBSET, MAPP2) .. 70
FIGURE 12 MPLONG CHANGE FROM PHASE 3 PARENT STUDY BASELINE IN CAPS-5 TSS (EFFECTIVENESS SUBSET MAPP1 AND MAPP2)-TIME SINCE TREATMENT SUBGROUPS 70
FIGURE 13: CHANGES IN ALCOHOL USE DISORDERS IDENTIFICATION TEST (AUDIT) 72
FIGURE 14: NUMBER OF PARTICIPANTS REPORTING THE PRESENCE OF SUICIDAL IDEATION AS MEASURED WITH C-SSRS AT EACH VISIT AND SEPARATED BY TREATMENT GROUPS [14] 75

List of Abbreviations

Ach Acetylcholine
AE(s) Adverse Event(s)
ALT Alanine Aminotransferase
ARF Acute Renal Failure
AVP Arginine Vasopressin
BDNF Brain Derived Neurotrophic Factor
bpm Beats per minute
°C Degrees Celsius
CAPS Clinician Administered PTSD Scale
CBCT Cognitive-Behavioral Conjoint Therapy
CBF Cerebral Blood Flow
CBT Cognitive Behavioral Therapy
CL Renal Clearance
CL/F Oral Clearance
cGMP Current Good Manufacturing Practice
CNS Central Nervous System
COMT Catechol-O-methyltransferase
C-SSRS Columbia Suicide Severity Rating Scale
CSO Concerned Significant Other
CTproAVP Stimulating Secretion of Copeptin
DAT Dopamine Transporter
DEA Drug Enforcement Administration
DBP Diastolic Blood Pressure
DMF Drug Master File
DNA Deoxyribonucleic Acid
DSM-5 Diagnostic and Statistical Manual of Mental Disorders, 5th Edition ED Emergency Department
EKG Electrocardiogram
EMDR Eye Movement Desensitization and Reprocessing
ESR Erythrocyte Sedimentation Rate
FAERS FDA Adverse Event Reporting System
FDA Food and Drug Administration
fMRI Functional Magnetic Resonance Imaging
G-CSF Granulocyte-colony Stimulating Factor
GD Gestational Days
HCl Hydrochloride
HHA 3,4-Dihydroxyamphetamine
HHMA 3,4-Dihydroxymethamphetamine
HMA 4-Hydroxy-3-methoxy-amphetamine
HMMA 4-Hydroxy-3-methoxy-methamphetamine
HPA Hypothalamus-pituitary-adrenal
HPMC Hydroxypropylmethylcellulose

HR Heart Rate
IB Investigator's Brochure
IL Interleukin
IMP Investigational Medicinal Product
IND Investigational New Drug
LD50 Lethal Dose in 50% of Cases
LSAS Liebowitz Social Anxiety Scale
LSD d-Lysergic Acid Diethylamide
LTFU Long Term Follow Up
MAO-A Monoamine Oxidase A
MAOI Monoamine Oxidase Inhibitor
MAPS Multidisciplinary Association for Psychedelic Studies
mCPP meta-Chlorophenylpiperazine
MDA 3,4-Methylenedioxyamphetamine
MDMA 3,4-Methylenedioxymethamphetamine
mmHG Millimeters of Mercury
MRI Magnetic Resonance Imaging
mRNA Messenger Ribonucleic Acid
MS Medication session
MW Molecular Weight
nAChr Nicotinic Acetylcholine Receptors
NET Norepinephrine Transporter
NK Natural Killer
OP Open Label
PASAT Paced Auditory Serial Addition Task
PET Positron Emission Tomography
PFC Prefrontal Cortex
PMA Paramethoxyamphetamine
PMMA Paramethoxymethamphetamine
PTSD Posttraumatic Stress Disorder
RACT Risk Assessment and Categorization Tool
RBANS Repeatable Battery for the Assessment of Neuropsychological Status RSI Reference Safety Information
SAE(s) Serious Adverse Event(s)
SAP Statistical Analysis Plan
SAR Serious Adverse Reaction
SBP Systolic Blood Pressure
SERT Serotonin Transporter
SIADH Syndrome of Inappropriate Antidiuretic-hormone Secretion
SNRI Serotonin and Norepinephrine Uptake Inhibitor
SPECT Single Photon Emission Tomography
SRR Spontaneous Reported Reaction
SSRI Selective Serotonin Reuptake Inhibitor

SUD Subjective Units of Distress
SUSAR Suspected Unexpected Serious Adverse Event
TEAE Treatment-emergent Adverse Event
TNF-**α** Tumor Necrosis Factor-alpha
URTI Upper Respiratory Tract Infection
VHD Valvular Heart Disease
VMAT2 Vesicular Monoamine Transporter 2
WBC White Blood Cell Count
5HT Serotonin
8-OH-DPAT 8-Hydroxy-2-(di-n-propylamino)tetralin

1.0 Summary

Mental health disorders are increasingly recognized as a leading cause of disease burden globally. From addiction to dementia to anxiety, almost one billion people worldwide suffer from mental disorders. Lost productivity because of two of the most common mental disorders, anxiety and depression, cost the global economy US$ 1 trillion each year. In total, poor mental health was estimated to cost the world economy approximately US$ 2.5 trillion per year in poor health. It reduced productivity in 2010, projected to rise to US$6 trillion by 2030[1]. In the United States of America, one in five adults' lives with mental illness (57.8 million in 2021).[2]

Treatment of mental illness depends on the type of disorder, its severity and what works best for the patient. In many cases, a combination of treatments is often the right answer. Mild mental illness with well-controlled symptoms, for example, might be managed by a patient's primary care provider. However, often, a team approach is appropriate to make sure a patient's medical, social, and psychiatric needs are met. This is especially important for severe mental illness or treatment-resistant mental illnesses, such as post-traumatic stress disorder (PTSD).

PTSD is an anxiety disorder that can develop after one experiences or witnesses a traumatic event involving actual or threatened death or harm or when one learns of someone else threat of actual harm (APA Diagnostic and Statistical Manual, 1994). PTSD is often comorbid with substance abuse, major depression, other anxiety disorders and suicidality. In more severe cases, often seen in veteran populations, psychotic features and increased resistance to treatment is evident[3]. Epidemiological studies estimate the lifetime prevalence of PTSD ranges from 6.1 to 9.2 percent in national samples of the general adult population in the United States and Canada, with one-year prevalence rates of 3.5 to 4.7 percent.[4]

The only US FDA-approved drugs for the treatment of PTSD are selective serotonin reuptake inhibitors (SSRIs), sertraline (Zoloft, Pfizer) and paroxetine hydrochloride (Paxil, GSK). All other agents, such as fluoxetine, venlafaxine, and paroxetine mesylate, are considered for off-label use. Veterans Affairs (VA) also reports the off-label use of nefazodone (serotonin reuptake inhibitor), imipramine (tricyclic antidepressant) and phenelzine (monoamine oxidase inhibitor) to manage PTSD[5]. Evidence-based psychotherapies and pharmacotherapies exist but may be difficult to tolerate and are ineffective for many individuals with PTSD, with an estimated 40-60% of patients remaining asymptomatic and meeting diagnostic criteria even after receiving treatment.

Novel interventions are therefore needed to treat people with mental illnesses better, especially those with PTSD and social anxiety. One such therapy has been under investigation by the Multidisciplinary Association for Psychedelic Studies (MAPS), a United States-based non-profit research and educational organization. Clinical trials of 3,4-methylenedioxymethamphetamine (MDMA) assisted therapy (MDMA-AT) for patients with chronic psychiatric disorders such as PTSD, social anxiety, and anxiety related to terminal illnesses were sponsored by MAPS.

In 2014, MAPS PBC (MAPS public benefit corporation) spun out of its parent organization (MAPS) and has been spearheading the clinical developmental effort of MDMA-assisted therapy for post-traumatic disorder (PTSD). MAPS PBC has completed two phase 3 trials. The first, a pivotal Phase 3 clinical trial, MAPP1, demonstrated that PTSD symptoms were significantly attenuated by MDMA-assisted therapy and confirmed the findings seen in Phase 2 studies. Manualized therapy in conjunction with MDMA was statistically superior for PTSD treatment in CAPS-5 severity scores from Baseline to 2 months after three blinded experimental sessions in comparison to therapy paired with an inactive placebo ($P<0.0001$, $d= 0.91$).

The second confirmatory, Phase 3 clinical trial (MAPP2), validated the previous results and significantly mitigated depressive symptoms as assessed using the BDI-II. Of note, MDMA did not increase the occurrence of suicidality during the study. Analysis of changes in CAPS (Clinician-Administered PTSD Scale) scores in previous studies showed effect sizes of 0.31 and 0.37 for sertraline compared to placebo and 0.56, 0.45, and 0.09 for paroxetine compared to placebo.[6–8] In stark contrast, MAPP 2 study results revealed an effect size of 0.91 for MDMA-assisted therapy versus placebo therapy, marking a significant improvement over any other PTSD pharmacotherapy previously identified.[9]

PharmAla Biotech is a Canadian Biotechnology company incorporated in British Columbia, Canada and licensed by Health Canada. The primary goal of PharmAla Biotech is to establish manufacturing of clinical grade 3,4-methylenedioxy-methamphetamine, also known as Laneo™ MDMA, in Canada and to support clinical trials being planned by several scientific researchers and clinicians across the globe. Laneo™ MDMA is manufactured, encapsulated, and packaged as per good manufacturing practices (GMP).
PharmAla Biotech is not the sponsor of the clinical trials; therefore, the information presented in this Investigator's Brochure (IB) summarizes results encompassing pharmacology and toxicology from published research studies of MDMA conducted by groups outside of PharmAla Biotech. This IB will provide investigators with the physical, chemical, and pharmaceutical properties of Laneo™ MDMA manufactured by PharmAla Biotech and the associated formulation(s) that will be made available to researchers and sponsors of clinical trials.

2.0 Introduction

MDMA is a ring-substituted phenethylamine also known as methylenedioxymethamphetamine. It was first synthesized and patented by Merck in 1912 and is currently not covered by a patent. In 1976, the renowned chemist Alexander Shulgin rediscovered MDMA and along with his colleagues, Shulgin published his experiences with 80mg to 160mg MDMA [10]. They reported that MDMA was found to influence human emotional status in a unique way without adversely affecting physiological functions or perception, such as visual perception or cognition.

The late Ralph Metzner proposed to call MDMA an "empathogen"; however, as Nichols explains, MDMA does not simply evoke empathy but in fact, enhances empathy. Therefore, the term "entactogen" was coined, composed of the Greek roots *en* and *gen* meaning *within* and *to generate*, respectively, and *tactus*, the Latin root for touching. Entactogens or MDMA-like compounds are substances that allow or promote a touching within or reaching inside to retrieve repressed memories[11]. Prior to its addition to Schedule 1 controlled substances list in the U.S. in 1985, MDMA was used to enhance therapy for individuals, couples, and groups to treat various psychological disorders, including moderate depression and anxiety.

MDMA is a chiral drug molecule; the hydrochloride salt of the racemic anhydrous MDMA is readily soluble in water and lipophilic once ionized. Estimates from animal data suggest a median lethal dose (LD50) in humans between 10 to 20 milligrams per kilogram (mg/kg). MAPS has sponsored several human trials using fixed doses equivalent to between 1 and 4 mg/kg MDMA HCl (active dose ranging from 75 mg to 225 mg). The onset of MDMA effect occurs 30 to 60 minutes after oral administration, peak effects appear 75 to 120 minutes post-drug, and the duration of effects lasts from 3 to 6 hours, with most effects returning to baseline or near-baseline levels 6 hours after drug administration. The elimination half-life of active doses of MDMA is 7 to 9 hours.

The pharmacokinetics of MDMA in humans has been characterized using oral doses of up to 150 mg MDMA, and disposition follows nonlinear pharmacokinetics. Metabolism of MDMA results in N-demethylation to 3,4-methylenedioxyamphetamine (MDA). The parent compound and MDA are further O-demethylated to 3,4-dihydroxymethamphetamine (HHMA) and 3,4-dihydroxyamphetamine (HHA), respectively. Both HHMA and HHA are subsequently metabolized to 4-hydroxy-3-methoxy-methamphetamine (HMMA) and 4-hydroxy-3methoxy-amphetamine (HMA). These 4 metabolites are known to be excreted in the urine as conjugated glucuronide or sulphate metabolites [12,13]

MDMA concomitantly promotes release, inhibits reuptake, and extends duration of serotonin, norepinephrine, and dopamine in the synaptic cleft to increase serotonergic, noradrenergic, and dopaminergic neurotransmission. MDMA produces anxiolytic and prosocial effects through release of the monoaminergic neurotransmitters, with the greatest effect on serotonin, followed by norepinephrine and dopamine. Subjective effects of MDMA can include increased compassion for self and others, reduced defenses, and fear of emotional injury, and making unpleasant memories less disturbing while enhancing communication and capacity for introspection. These factors taken together provide the opportunity for a corrective emotional experience in the context of therapy. Many of the therapeutic effects of MDMA-assisted therapy are evident within a short period of treatment, often after the initial session. Increased feelings of interpersonal closeness, changes in social perception, and reduced anxiety might make MDMA a suitable pharmacological adjunct to enhance therapy for treatment of anxiety disorders, such as PTSD, social anxiety, and anxiety associated with other conditions.

MAPS completed a pivotal Phase 3 clinical trial (MAPP1) in 2020 demonstrating the efficacy and safety of MDMA-assisted therapy for treatment of PTSD [14]. In this randomized, double-blind, placebo-controlled, multi-site study, participants with treatment resistant PTSD were treated using divided doses of 80 mg to 180 mg MDMA HCl. Therapy in conjunction with MDMA was statistically superior for PTSD treatment as measured by a decrease in CAPS-5 severity scores from Baseline to 2 months after three blinded dosing sessions in comparison to therapy paired with an inactive placebo. These findings were supported by previous Phase 2 clinical trials conducted to develop the medical use of MDMA-assisted therapy for patients with chronic PTSD. The US FDA granted MDMA Breakthrough Therapy Designation in 2017, accelerating its therapeutic development based on substantial placebo-subtracted effect sizes, indications of efficacy in a Phase 3 trial, and favourable safety outcomes for the treatment of PTSD. The most prevalent Treatment-emergent Adverse Events (TEAEs) related to MDMA in the pivotal Phase 3 trial MAPP1 were muscle tightness, decreased appetite, nausea, hyperhidrosis, and feeling cold. Safety findings from MAPP1 were comparable to the Adverse Events (AEs) profile reported in the pooled Phase 2 analysis. Most AEs were mild to moderate in severity, lasted no longer than 7 days, and did not require any treatment.

MAPS has completed several Phase 2 and one Phase 3 investigations of MDMA-assisted therapy for the treatment of PTSD[15]. The pooled Phase 2 efficacy results in PTSD participants indicate that MDMA-assisted therapy administered in a controlled clinical setting demonstrated that the treatment was safe and efficacious among patients with moderate to severe PTSD[16,17]. Durable improvements in PTSD symptom severity were found at least 12 months after the last dosing session in 91 participants who received a therapeutically active dose of MDMA in these studies, and 67% did not meet PTSD diagnostic criteria per CAPS-4 assessment. Though the interpretation of long-term results might be limited due to a lack of a blinded control group at this time point, the results suggest significant, durable improvement in PTSD symptoms that lasted for at least 12 months for many participants following MDMA-assisted therapy[16].
The findings from two open-label studies (MP16 and MP17), testing the modality of MDMA-assisted therapy in PTSD in two multi-site clinical trials to assess the feasibility of scaling across 14 North American sites demonstrated that the observed treatment response to MDMA-assisted therapy can be scaled and replicated by newly trained therapy teams across multiple sites. .

The ongoing studies by Lykos Therapeutics (previously known as MAPS PBC) and its collaborators include a) pilot Phase 3 trial to examine the preliminary effectiveness of MDMA-facilitated brief cognitive conjoint therapy for improving chronic PTSD and relationship functioning in a sample of veterans (NCT05979844); b) an observational trial biomarker study designed to characterize how human neural circuits and behaviours are modified during altered states induced by MDMA (NCT04060108) and c) a Phase 1 open-label study to evaluate the effect of MDMA in subjects with moderate hepatic impairment and subjects with normal hepatic function (NCT03606538).

As per data from MAPS studies and other researchers' published literature, MDMA produces sympathomimetic effects that include transient, self-limiting increases in heart rate (HR) and blood pressure that appear to be well-tolerated by healthy volunteers. Similar transient effects were observed in Phase 2 and Phase 3 studies in participants with PTSD. Most people did not experience elevation that exceeded those seen after moderate exercise. Risks posed by elevated blood pressure were addressed by excluding candidates with a history of cardiovascular and cerebrovascular disease or pre-existing uncontrolled hypertension and by regularly monitoring blood pressure and pulse throughout dosing sessions [14]. Typical reactions from MDMA reported in the literature and clinical trials appear to be transient and diminish as drug effects wean during the session and over the next one to seven days.

Risks posed by sympathomimetic effects of MDMA treatments were addressed in MAPS clinical trials by excluding people with pre-existing underlying cardiovascular disease or uncontrolled hypertension and by monitoring blood pressure, body temperature, and pulse before each dose and at the end of experimental sessions. MDMA may reduce responsiveness to water/salt balance changes after normal and increased water consumption. MDMA is also a mild immunosuppressant. Further, drug administration was only conducted under direct observation in a community-based healthcare setting, and no take-home doses were permitted.

3.0 Physical, chemical and pharmaceutical properties of Formulation

MDMA is the short form of the name 3,4-methylenedioxymethamphetamine. The International Union of Pure and Applied Chemistry (IUPAC) nomenclature is (RS)-1-(benzo[d][1,3]dioxol-5-yl)-N-methylpropan-2-amine, and the United States Adopted Name (USAN) is Midomafetamine hydrochloride. PharmAla Biotech has further trademarked the name and refers to racemic MDMA as Laneo™ MDMA. .

Laneo™ MDMA is structurally like but functionally distinct from amphetamines and mescaline. Laneo™ MDMA, also known as 3,4-methylenedioxy-N-methylamphetamine and N-methyl-3,4-methylenedioxyamphetamine, has the chemical formula of $C_{11}H_{15}NO_2$. MDMA is a chiral molecule, possessing two enantiomers, S(+)-MDMA and R(-)-MDMA, with S(+)-MDMA being more potent than R(-)-MDMA[11]

Research in humans to date and most nonclinical studies have used racemic MDMA or an admixture containing equal amounts of both enantiomers. The current manufacturing process uses achiral methods and produces racemic MDMA.

The hydrochloride salt of MDMA is readily water soluble with a pKa of 9.9. The purity of Laneo™ MDMA is established at 98% -102%, with total impurities capped at not more than 2% and water at not more than 0.5%.

Up to twenty-four months of stability data at long-term storage conditions of 25°C/60%RH and up to six months of accelerated storage conditions at 40°C/75%RH are available on the drug substance. No significant degradation has been detected under these conditions, establishing an interim re-test period of twenty-four months. A batch of MDMA HCl is used to manufacture the investigational medicinal product.

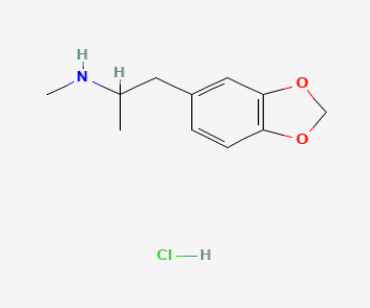

Figure 1: Chemical structure of Laneo(TM) MDMA HCl

MDMA (as the hydrochloride salt) in the form of off-white crystalline powder is compounded with inactive ingredients. The blend is then encapsulated in gelatin capsules containing 40mg MDMA HCl. A matching inactive placebo is manufactured to support placebo-controlled studies.

Stability data is on the manufactured product, designed in compliance with the ICH Q1A R2, ICH Q6A, and ICH Q1E, up to 6 months under accelerated storage conditions, 40°C/75%RH, up to 24 months under long-term storage conditions 25°C/60%RH and intermediate storage conditions 30°C/75%RH, in high-density polyethylene bottles with a child-resistant closure.

In June 2023, PharmAla released a clinical batch of the investigational medicinal product (IMP) at the dosage strength of 40mg MDMA HCl per capsule, manufactured according to current Good Manufacturing Practices (cGMP) by a certified site.
Six months of accelerated stability data at 40°C/75%RH and eighteen months of long-term storage conditions at 25°C/60%RH are available on the drug product. No significant degradation has been detected under any of these conditions. An interim re-test period of two years when stored at ambient storage conditions (between 15°C and 25°C) has been established. As data from the ongoing stability program becomes available, the re-test and shelf-life will be amended appropriately. .
In November 2023, a second batch of drug product was manufactured and released.

Based on existing knowledge in the public domain and equivalency in the manufacturing process employed by PharmAla Biotech to already established processes [18], it is estimated that the 40mg MDMA HCl capsules will have a shelf life of at least 36 months with recommended storage conditions at ambient temperatures up to 30°C [15].

As a Schedule I controlled substance, MDMA is stored and handled in compliance with relevant federal or national, state, provincial and local regulations. License holders are

responsible for administering and dispensing the MDMA for approved uses only. They are responsible for ensuring that it is stored securely in accordance with the requirements of the local and other relevant international regulatory authorities.

4.0 Nonclinical Studies

4.1 Nonclinical Pharmacology

A summary of findings from nonclinical animal and in vitro research published in the public domain is presented.

Research into the pharmacological, physiological, or psychological effects of MDMA began in the 1950s, when the U.S. Army administered MDMA to guinea pigs, monkeys, mice, rats, and dogs as part of a military research program, possibly intending to develop chemical incapacitants or means of enhancing interrogation. Investigations of the pharmacology, functional effects and toxicity of MDMA in animals have generally included injections of large and often-repeated doses of MDMA that are not human-equivalent doses. Studies of MDMA have been conducted in primates and rodents. Primate species studied include baboons, macaque, rhesus monkeys, and squirrel monkeys, and rodents include mice and rats. Studies of circadian rhythm have occurred in hamsters. Beginning in the mid-2000s onwards, reports re-examining these effects have questioned the applicability of allometric interspecies scaling models for MDMA [15].

MDMA possesses a complex pharmacological profile dominated by its effects as a monoamine releaser and reuptake inhibitor. Its prominent serotonergic effects differentiate it from amphetamine and methamphetamine, which primarily act on dopamine and norepinephrine. MDMA also has a complex profile from a pharmacokinetics perspective and has been demonstrated in multiple animal models to follow nonlinear pharmacokinetics, with increased doses resulting in a disproportionate increase in exposure to the parent compound. Additionally, MDMA exhibits pharmacodynamic drug interactions with other drugs commonly used in psychiatry[19–21]. A summary of nonclinical toxicology studies conducted by MAPS is available below in Table 1..

Table 1: Summary of Nonclinical toxicology studies available in the literature[15]

Species	Type of Study	Findings (when available) and Notes
Rat (Sprague-Dawley)	Single- and 13-Day Repeat dose Oral Toxicity Study	Marked adverse clinical reactions (>25 mg/kg), including death (150-300 mg/kg), and blood chemistry changes suggestive of liver and kidney damage. No histologic evidence of brain damage seen with standard H&E or nervous system-specific stains.
Rat (Sprague-Dawley)	4-Day Repeat-Dose Oral Neurotoxicity Study	Persistent decrease in 5-HT and 5-HIAA at both doses at 2- and 4-weeks post-dose. Reversible decrease in HVA at 80 mg/kg.
Rat (Sprague-Dawley)	28-Day General Toxicology	↓ weight gain
		↓ ♀ urine pH, blood: BUN, Glu, Creatinine, LDH, (♀,♂) Cl
		↑ (♀,♂) WBC, Phos (trend)
Dog (beagle)	28-Day General Toxicology	↓ weight gain 9, 15 mg/kg
		↓ testicle size
		↑ prostate size
		Deaths at 15 mg/kg
In vitro bacteria	†Genotoxicity Ames test	Negative for mutagenic activity ± metabolic activation
In vitro CHO cells	†Genotoxicity Chromosome Aberration test	Negative for clastogenic activity *in vitro* ± metabolic activation
Rat (Sprague-Dawley)	†Genotoxicity *In vivo* Micronucleus Evaluation by Oral Gavage	Negative for clastogenic activity and disruption of mitotic apparatus *in vivo* at MTD 100 mg/kg/day x 2 days, single housing
In vitro HEK-hERG	†hERG Channel Inhibition patch clamp assays per ICH S7A/S7B	Negative: IC50 of 206 µM (~170x over clinical Cmax); Hill coefficient=1.1
Rat (Sprague-Dawley)	†Pilot Prenatal Developmental Toxicity Study	Well tolerated by time-mated ♀ at 20 mg/kg/day p.o. GD6-17, no effect on ovarian/uterine implantation, mean gravid uterine weights, or fetal body weights. No external malformations or developmental variations in any fetus at GD21 TK: No systemic accumulation with repeated daily doses
Rabbit (New Zealand White)	†Pilot Prenatal Developmental Toxicity Study	Well tolerated by time-mated ♀ at 10 mg/kg/day p.o. GD7-19, deaths at 15 and 20 mg/kg/day. At tolerated dose, no effect on ovarian/uterine implantation, mean gravid uterine weights, or fetal body weights. No external malformations

		or developmental variations in any fetus at GD29. TK: systemic accumulation with repeated daily doses
Rat (Sprague-Dawley)	†Reproductive Toxicology:	NOAEL dose 10 mg/kg/day p.o. for maternal, paternal reproductive performance and fertility
	Fertility/Early Embryonic Development Study	Mortality at (n=1,♂) D57, (n=1,♀ euthanasia due to tail injury) D15 10 mg/kg/day [not MDMA-related]
Rat (Sprague-Dawley)	†Reproductive Toxicology: Embryo-Fetal Development Study	NOAEL 15 mg/kg/day p.o. for maternal and developmental toxicity at GD17 Cmax 1330 ng/mL, AUC0-24hr 10900 hr*ng/mL
Rabbit (New Zealand White)	†Reproductive Toxicology: Embryo-Fetal Development Study	NOAEL 10 mg/kg/day p.o. for maternal and developmental toxicity at GD19 Cmax 603 ng/mL, AUC0-24hr 2920 hr*ng/mL
Rat (Sprague-Dawley)	†Extended Single-dose 28-Day Neurotoxicology Study	(♀) NOAEL 25 mg/kg/week p.o.
		Day0-22 Cmax 1490 ng/mL, AUC0-10hr 10700 hr*ng/mL
		(♂) NOAEL 20 mg/kg/week p.o.
		Day0-22 Cmax 1100 ng/mL, AUC0-10hr 4590 hr*ng/mL
		Mortality at 25 mg/kg (n= 2 ♂) on Day 2 & (n= 4 ♂) on Day 16
		No MDMA-related CNS neurotoxicity observed in expanded neurohistopathology through MTD
		Myofiber degeneration in skeletal muscle associated with mononuclear cell and neutrophil infiltration
		↓ (♂) food consumption 25/20 mg/kg Wk 2
		↓ (♂) weight gain 25/20 mg/kg Wk 1-2
		↓ (♀) weight gain 25/20 mg/kg Wk 1
		Clinical observations were transient, trending toward resolution 24 hrs post minimal subclinical dehydration
		↑ salivation, red material around nose (♂) ≥ 7.5 mg/kg, (♀) 25 mg/kg,
		↑ activity, hypersensitivity to touch (♂) 25/20 mg/kg (♀) ≥ 7.5 mg/kg,
		↑ (♀) hypersensitivity to sound and piloerection 25mg/kg,
		↑ (♂) aggression 25/20 mg/kg,
		↑ (♂,♀) stereotypy 25/20 mg/kg
		↑ (♂) penile protrusion, anogenital swelling +/- sperm plug (n=2) 25 mg/kg D1
		TK: Cmax approx. dose proportional, AUC0-10hr > dose proportional

		No systemic accumulation or reduction with repeat weekly doses, no sex effect
		Dose includes HCl correction factor
Dog (beagle)	†Extended Single-dose 28-Day Neurotoxicology and In Vivo Cardiovascular Study	(♀, ♂) NOAEL 4 mg/kg/week p.o.
		Day 0-22 Cmax 219 ng/mL, AUC0-8hr 983 hr*ng/mL
		Mortality at 9 mg/kg (n=1 ♀) & 12 mg/kg (n=4: n= 2 ♀, 2 ♂) on Day 1
		No MDMA-related neurotoxicity observed in expanded neurohistopathology through MTD ↓
		(♂) body weight 12/9 mg/kg/week Wk 1, ↓ (♀,♂) weight gain through Wk 4 ≥ 2 mg/kg/week
		Clinical observations were transient, trending toward resolution 24 hrs post dose:
		↑ (♀,♂) salivation, injected sclera, hypersensitivity to touch, emesis ≥ 2 mg/kg/week
		↑ (♀,♂) activity ≥ 4 mg/kg/week
		↑ (♀,♂) dilated pupils, aggression, hypersensitivity to sound, vocalization, trembling, closed eyelids, stereotypy, audible breathing, coughing, cold skin 12/9 mg/kg/week
		↑ (♀) ocular discharge (n=1) 12 mg/kg D2
		TK: Cmax & AUC0-8hr appr. dose proportional, no systemic accumulation with repeat weekly doses, no sex effect
		ECG: no effect on QT/QTc interval
		Dose includes HCl correction factor
† =Studies conducted by Charles River Labs		

4.1.1. Primary Pharmacodynamics

Most effects of MDMA likely arise directly from monoamine reuptake inhibition and release and indirectly from activation of downstream monoamine receptors and subsequent secretion of neuromodulators oxytocin and arginine vasopressin (AVP). MDMA binds primarily to membrane-bound monoamine transporters, which remove monoaminergic neurotransmitters from the space between neurons, known as the synaptic cleft. MDMA appears to alter the conformation of the transporters, enabling monoamines to diffuse out of the neuron rather than being actively transported into the presynaptic neuron. MDMA prevents the reuptake of serotonin and, to a lesser extent, norepinephrine and dopamine and facilitates the release of these neurotransmitters. The selectivity of MDMA for specific monoaminergic neurotransmitters is species-dependent and cannot solely be attributed to differences in binding affinity for specific reuptake transporters observed in vitro, as described below. In in vitro studies, MDMA was also found to compete with monoamines for sites on the vesicular

monoamine transporter-2 (VMAT2), suggesting MDMA also promotes active release of monoamines from vesicular stores, in addition to inhibiting reuptake [15].

MDMA can inhibit monoamine oxidase A (MAO-A) in vitro at high concentrations, which preferentially degrades serotonin, and leads to accumulation of extracellular serotonin in the synaptic cleft[22–25]. Inhibition of MAO-A may have played a role in fatalities and medical emergencies seen after combining Ecstasy with monoamine oxidase inhibitors (MAOI) in epidemiological settings[26–28].

The ability of MDMA to stimulate the release of pre-synaptic serotonin, norepinephrine, and dopamine in multiple brain regions and inhibit reuptake has been well documented[29]. *In vivo* microdialysis and voltammetry results show significant enhancement of serotonin and to a lesser extent, dopamine following MDMA administration, a response attenuated by various transporter inhibitors. MDMA-stimulated serotonin and dopamine release has been measured in the striatum, nucleus accumbens, prefrontal cortex (PFC), and hippocampus of freely moving rats, including after administering 0.32 to 3.2 mg/kg MDMA HCl. The subjective and physiological effects of MDMA are produced by the dynamic interaction of these transmitter systems on numerous brain networks that modulate learning and memory, emotion, reward, attention, sympathetic/parasympathetic activity, and neuroplasticity [15].

Findings from drug discrimination studies in rats suggest dose-dependent differences in the role of the serotonergic versus the dopaminergic system, with rats trained on 1.5 mg/kg MDMA HCl recognizing Selective Serotonin Reuptake Inhibitors (SSRIs) as similar while rats trained on 3.0 mg/kg recognized amphetamine as similar, and rats trained on both doses recognizing 5HT1A-related compounds as similar. Training with 1.5 mg/kg but not 3.0 mg/kg MDMA HCl resulted in considering higher doses of a 5HT2A agonist as similar[30]. The same research team determined that dopamine antagonists interfered with the stimulus properties of amphetamine but not MDMA [15].

4.1.2 Secondary Pharmacodynamics

MDMA has been shown to have diverse secondary pharmacodynamics in animals. Both enantiomers of MDMA enhance Ach release in the PFC and promote changes in GABAergic systems that correlate with sociability. There is some evidence that 5HT2B receptors are involved in stimulating increased locomotor activity in mice, reported in studies administering 20 mg/kg [31]. At least some direct or indirect effects of MDMA on serotonin receptors may alter GABA uptake in the ventral tegmental area of rats. An in vitro study found that S-MDMA showed signs of competitive interaction with the alpha-4 beta-2 nicotinic receptor, which is implicated in learning, while R-MDMA did not produce this effect. [32]

In rats, 10 or 20 mg/kg doses of MDMA HCl elevated serum corticosterone (a rodent cortisol analog) and prolactin, with elevations lasting up to 4 hours after dosing and with hormone levels attenuated by a 5HT2A serotonin receptor antagonist[33,34]. Given the dosage used was

five to 10.7 times larger than an active dose in humans, it is unclear if this response is analogous to elevated cortisol in humans or whether it reflects a different process. Administering 1 to 3 mg/kg doses found that R (-)-MDMA, but not S(+)-MDMA, significantly increased prolactin levels in rhesus monkey plasma, suggesting that at least the R(-) enantiomer of MDMA can influence endocrine signalling at doses relevant for studies in humans [34]. Fluoxetine attenuated prolactin release after administration of racemic MDMA, and fluoxetine and a 5HT2A antagonist attenuated prolactin release after R (-)-MDMA, indicating that prolactin release is associated with serotonin release and indirect action on 5HT2A receptors by R (-) -MDMA [34].

MDMA has been shown to have significant effects outside the central nervous system; namely on the cardiovascular, osmoregulatory, and immune systems. MDMA has been shown to cause increases in blood pressure and heart rate in small mammals and primates. These effects are possibly controlled through increased sympathomimetic activity via beta-adrenergic receptors. MDMA has also been shown to effect water regulation by activation of the AVP system, thus explaining the thirst seen in humans.

4.1.3 Safety Pharmacology

Safety pharmacology studies are those studies that investigate a substance's potential undesirable pharmacodynamic effects on physiological functions in relation to exposure in the therapeutic range & above. The safety pharmacology core battery aims to investigate the test substance's effects on vital functions.

Intravenous MDMA doses that cause lethality in 50% of the cases, known as the LD50, are 97 mg/kg MDMA HCl in mice, 49 mg/kg in rats, 14 to 18 mg/kg in dogs, and 22 mg/kg in monkeys (24)This section will summarize some of the literature available in the public domain along with data from the studies sponsored by MAPS. The investigator is encouraged to refer to the original publications for a more detailed understanding of the MAPS-sponsored data[15].

4.1.3.1 Central Nervous System

In a MAPS-sponsored pilot repeated dose 13-day toxicity study (EMD-AT-001), testing increasing doses ranging from 25 mg/kg to 300 mg/kg MDMA HCl, no changes were found in the brain tissue of treated rats compared to controls. The subsequent definitive GLP 28-day repeated dose toxicity study in rats found vacuolar changes in the brainstem adjacent to the trigeminal nuclei in 1 of 10 animals receiving 50 mg/kg/day of MDMA HCl and similar changes in 5 of 10 male rats receiving 100 mg/kg/day. No changes were observed in female animals.

The definitive 28-day repeated dose toxicity study in dogs also found possible changes in the CNS of MDMA-treated dogs with doses of 3, 9, and 15 mg/kg/day; however, the effect was not dose-dependent, suggesting that they could be background events. These possible

changes included white matter changes, neural malacia, and cellular infiltrates of the cerebrum. Neural chromatolysis was observed in the brainstem. The pathogenesis of these changes was unknown. Serotonergic axons can resist silver staining, providing an alternate explanation for these inconclusive findings.[15]

A MAPS-sponsored follow-up repeat-dose neurotoxicity study administering Sprague-Dawley rats 40 mg/kg or 80 mg/kg of p.o. MDMA HCl twice daily for four days showed no morphological brain changes (EMD-SC-003). Neurochemical brain changes related to MDMA administration included a 50% decrease in serotonin (5-HT) and 5-Hydroyindoleacetic acid (5-HIAA) at both dosing levels. This decrease was apparent 2 and 4 weeks after exposure. A temporary 34% decrease in homovanillic acid (HVA) was observed in the 80 mg/kg group 2 weeks after treatment. Four weeks after treatment, the HVA levels had returned to normal. These findings indicate that repeated high-dose administrations of MDMA 2x per day over four days produce long-term reductions up to 4 weeks later in 5-HT and 5-HIAA in the rat while having no apparent effect on the dopaminergic system.

MAPS has sponsored definitive GLP Extended Single-dose neurotoxicity studies in rats and dogs, found no evidence of CNS lesions at doses tested, 0 to 25mg/kg per oral MDMA in Rats and 0 to 12 mg/kg in beagle dogs, based on expanded neuro histopathology through the maximum tolerable dose. An interim necropsy was performed on selected animals on Day 2 to examine single-dose effects, and the remaining animals were necropsied on Day 29, 7 days after the last dose, to examine repeat-dose effects. Based on this, the risk of CNS neurotoxicity with the intended clinical dosing regimen is postulated to be minimal[15].

Motor Effect

MDMA produces some repetitive behaviour in rodents, but not to the same degree as psychostimulants. MDMA has been shown to increase locomotor activity.[35–37] Rats on MDMA walk around a cage perimeter interpreted as an indicator of thigmotaxis, which is a sign of anxiety. Increased locomotion in rodents after MDMA may be regulated in part by 5HT2C and 5HT2B receptors, possibly through indirectly regulating dopamine and serotonin release, and at least one study reported that blocking alpha1 adrenergic receptors reduced locomotor activity after 5 mg/kg MDMA HCl. Rhesus monkeys did not exhibit increased locomotor activity after receiving up to 2.4 mg/kg MDMA HCl[38].

Behavioral Effects

Several dose-dependent differences on behavioral tests in rats given MDMA have been reported, including increased anxiety-related behaviors thought to be associated with serotonin syndrome, and decreased social anxiety at 5mg/kg i.p A biphasic dose response is seen with increasing doses of MDMA HCl in rats, increased anxiety in the elevated plus-maze at 7.5mg/kg and reduced anxiety at 15mg/kg[15]. Preclinical data in animals suggest that the profile of neurotransmitter release observed after MDMA administration may increase the risk

of mania in some individuals. However, mania has not been reported as a side effect of MDMA or Ecstasy in humans. Conflicting findings on the anxiogenic and anxiolytic dose-dependent effects of MDMA have been reproduced in clinical trials[15].

Morley and colleagues observed rat behaviour after receiving 5 mg/kg MDMA HCl, noting that the dose administered correlated with prosocial behaviour, such as lying next to each other[39–41] Subsequent studies suggest that MDMA increases prosocial behaviour in rats by elevating oxytocin in the paraventricular nucleus through 5HT1A receptor agonism, with the oxytocin increase arising from the indirect effects of MDMA on 5HT1A receptors via serotonin release. There have been no human pharmacological challenge studies combining MDMA with 5HT1A agonists, while 5HT1A antagonists have negligible effects on the subjective or physiological effects of MDMA in humans. As a result, it is unclear whether the rat behaviour is analogous to human reports of increased feelings of empathy or interpersonal closeness while under the influence of MDMA. Pitts and colleagues reported observing greater prosocial effects of MDMA when compared with the psychostimulant methamphetamine in squirrel monkeys, confirming rodent findings in primates[42]. The effects were seen with racemic MDMA and with each enantiomer, and the administration of a 5HT2A receptor antagonist dampened them. MDMA appears to have prosocial effects on animals less closely related to humans, including octopuses and zebrafish. The study in octopuses examined the octopus serotonin transporter gene, determining that the receptor that binds MDMA is conserved across species.[15]

Body temperature

Rodents have generally been used to study the hyperthermic effects of MDMA. Rodents have a much smaller body mass and do not sweat but use their tail to regulate body temperature. It has a large surface-to-volume ratio and is perfused with many blood vessels for thermoregulation. MDMA doses that are moderate to high elevate body temperature and disrupt thermoregulation in mice, and doses of MDMA HCl in the 1 to 2 mg/kg range cause a slight increase in body temperature. Rats given doses of 10 mg/kg MDMA HCl (s.c. and i.p.), but not 2 mg/kg, experienced increases in body temperature correlated with levels of the active metabolite MDA [43,44]. MDMA effects on body temperature are susceptible to changes in ambient temperature in rodents, with high ambient temperature significantly increasing body temperature in mice and rats and low ambient temperatures reducing it[15]; this should be considered when conducting human trials.

High doses of MDMA also produce significant elevations in body temperature in primates. At doses closer to those humans ingest (75 -125mg), monkey exhibits only slight to moderate elevation in body temperature. As per data from MAPS and other studies, findings in rodents do not extrapolate well to primates in this area. Given that the thermoregulatory effects in rodents are highly dose-dependent, most physiological effects in humans seen after MDMA administration suggest that a controlled environment and moderate doses are sufficient to mediate physiological complications associated with hyperthermia, including cardiovascular, osmoregulatory, neurological, and immunological effects[15]

4.1.3.2 Cardiovascular system

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), through its safety pharmacology guidelines S7A and S7B, mandates the testing required to demonstrate the safety of the drug substance and product. MAPS reported the results from the *in vitro* IKr Assay (commonly referred to as human Ether-a-go-go-Related gene (hERG) assay) demonstrate that MDMA has minimal risk of QTc prolongation or *Torasade de pointes (TdP)*. In comparison, SSRIs are known to carry low to moderate risk of QTc prolongation, including available PTSD medications paroxetine and sertraline.

MAPS has also completed an *in vivo* cardiovascular sub-study as part of the GLP Extended Single-dose neurotoxicity study, administering beagle dogs with 0 to 12mg/kg MDMA once weekly for four weeks. All animals in all groups received an electrocardiographic (ECG) examination pretest, and all survivors pre-dose and 1-2 hours post-dose on Day 1 and before terminal necropsy (study days 2 and 27). MAPS concluded there was no effect of the oral administration of MDMA on qualitative or quantitative ECG parameters in this study[15].

In vivo assessments of cardiovascular effects of MDMA in animals detected increased sympathomimetic activity, as seen in humans.[45] Interestingly, a biphasic response of diastolic blood pressure has been shown, with an initial pressor response (with two components and mainly mediated by **α**1-and **α**2-adrenoceptors) that is followed by a sustained depressor response mediated by **α**2-adrenoceptors.[45] In fact, this type of pressor response has been suggested to depend on the dose, as a dose of 5 mg/kg produced a biphasic response, whereas a dose of 20 mg/kg produced a pressor response. Given the affinity of MDMA for the NET, it is possible that the cardiovascular effects of MDMA could be partially attributed to norepinephrine signalling in the peripheral nervous system[25,45–47].

4.1.3.3. Respiratory System

MDMA causes serotonin reuptake inhibition and carrier-mediated release of serotonin which causes indirect agonism of serotonin receptors and has known sympathomimetic effects. Animal studies show that serotonin agonism increases respiratory drive via actions at 5HT1A, 5HT7, and 5HT4A. MDMA does not have detectable affinity for the mu opioid receptor ([15]) MAPS sponsored GLP 28 -day repeat dosing toxicology study in rats and dogs found no effect of MDMA on respiration rate.

4.1.3.4 Hepatic System

MDMA is primarily processed through the hepatic route, with 50 to 75% being metabolized. Standard toxicity studies failed to find liver damage after MDMA administration in rats or dogs after 28 days of daily exposure.[48] In a MAPS-sponsored pilot dose range finding study of

Sprague Dawley rats, toxicity was assessed in a 13-day increasing dose regimen, from 25mg/kg to 300mg/kg p.o. This study found significant elevations of the liver toxicity markers alanine aminotransferase (ALT) and aspartate transaminase (AST) in male rats. Despite a rise in these markers, gross pathology and microscopy of liver tissue failed to show any liver damage.

In the MAPS-sponsored definitive GLP 28-day repeated dose study of Sprague-Dawley rats, toxicity was assessed in a single-dose arm after 25 mg/kg p.o. MDMA (dose includes HCl correction factor). A marked increase in AST and ALT with a mild increase in alkaline phosphatase (ALP) and total bilirubin was found in a single animal that was found recumbent with decreased activity and hunched posture on Day 2 and subsequently euthanized, 24 hours post-dose. These were consistent with a hepatobiliary effect and correlated with the microscopic finding of liver necrosis. Moderate increases in triglyceride and cholesterol also indicate a lipid effect. Microscopically, there was extensive acute liver necrosis that was the likely proximate cause of death. Otherwise, there were no MDMA-related effects on gross findings or organ weights in the single-dose Day 2 or repeat-dose Day 29 animals in pathology or organ weight assessments for the remaining animals. Although direct hepatoxic effects are unlikely, MDMA carries a rare risk of idiosyncratic hepatotoxicity, which is also seen in other marketed medications[15].

4.1.3.5. Renal System

Renal clearance of MDMA is between 8% to 11% of the parent compound. All metabolites of MDMA in urine are detected as glucuronide and sulphate conjugates. Vasopressin, also named arginine vasopressin (AVP) is the main hormone responsible for water maintenance in the body through the antidiuretic actions in the kidney. The posterior pituitary into the blood releases vasopressin formed in the hypothalamus. Hypothalamic osmotic neurons are responsible to initiate the cascade for AVP actions[49].

In the MAPS Sponsored 28-day repeated dose studies, the only renal change observed microscopically was mild to minimal hydronephrosis, which the researchers attributed to treatment-related polyuria (abnormally large urine production). Gross pathology revealed enlarged urinary bladders (most likely polyuria-related) in some animals and was deemed potentially treatment-related. MDMA can influence water regulation by activation of the AVP system, as shown in several animal studies. A study of isolated in vitro rat hypothalamus initially reported AVP and oxytocin release after MDMA and its metabolite HMMA. *In vivo*, drug-discrimination studies in rats suggest that AVP receptors are involved in producing interoceptive effects of MDMA[15]. These findings should be taken into consideration when planning clinical trials.

4.1.4 Pharmacodynamic Drug Interactions

MDMA has been shown to interact with numerous CNS active compounds in animals, with effects on behavior, neurotransmitters, gene expression, and thermal regulation among others. Caffeine (10 mg/kg) has also been shown to increase MDMA-associated hyperthermia in rats when given with 2.5 mg/kg MDMA HCl. The increased hippocampal 5-HT loss was also seen in the caffeine-treated animals, which may result in increased MDMA toxicity [50–52].

MDMA modulates serotonin neurotransmission, therefore there is a potential for drug-drug interactions with medications that modulate the serotonin system, including SSRI, SNRIs, TCAs, MAOIs, atypical antipsychotics and others. Some of these interactions may increase the risk of toxidromes such as serotonin syndrome. Another pharmacodynamic interaction that may occur is the weakening of MDMA's subjective effects.

SERT inhibitors (SSRIs), including citalopram, paroxetine, and fluoxetine, reduce MDMA's physiological and subjective effects broadly. NET-specific inhibition by reboxetine reduced MDMA's NE-mediated cardio-stimulant properties and some psychological outcomes, while combined NET and SERT inhibition by duloxetine broadly attenuated physiological and psychological outcomes, like outcomes by SSRI+MDMA combinations [21]. Overall, monoamine reuptake inhibitors resulted in attenuation of MDMA's physiological and subjective effects, even in instances of higher MDMA plasma levels by means of CYP2D6 inhibition. This consistent outcome may suggest that, when administered together, monoamine reuptake inhibitors outcompete MDMA by binding to monoamine transporters (i.e., SERT, NET, DAT), preventing the efflux of monoamines into the synaptic cleft, thus attenuating the effects of MDMA. Exceptions to this trend include the combined NET and DAT inhibitor bupropion, which prolonged the subjective effects and heightened the positive mood effects of MDMA [21]

4.2 Pharmacokinetics and Product Metabolism in Animals

MDMA's pharmacology is complicated by non-linear pharmacokinetics observed in multiple mammalian models. Due to the nonlinear characteristics of MDMA, bioavailability is likely incomplete and variable across doses. MDMA has been shown to be primary eliminated by hepatic metabolism with minimal renal contribution in animals. MDMA has also been shown to have pharmacokinetic drug interactions with many drugs used in psychiatry, as both the perpetrator and victim compound due to its metabolism through and auto-inhibition of CYP2D6 (in humans), a common metabolizing enzyme of many CNS agents. Additionally, MDMA has been shown in nonclinical studies to have a large volume of distribution and high CNS penetration.

A summary of the pharmacokinetics of MDMA across multiple mammalian species is available in Table 2

Table 2: Pharmacokinetic constants for Plasma MDMA after various routes of administration to humans or animals.[15]

MDMA HCl	Cmax (ng/ml)	AUC (h•ng/ml)	Tmax (h)	t1/2 (h)
Rat				
2 mg/kg i.p.	210±108	163±56	0.14 ± 0.08	0.80 ±0.16
2 mg/kg s.c.	196±50	304±65	0.75 ± 0.29	0.79±0.14
2 mg/kg p.o.	46±15	61±42	0.56 ± 0.31	0.77±0.11
10 mg/kg i.p.	2257±131	3432±278	0.13 ± 0.04	1.08±0.14
10 mg/kg s.c.	1130±138	3146 ±514	1.10 ± 0.22	1.27±0.39
10 mg/kg p.o.	966±49	2226±301	0.31±0.13	1.62±0.41
2.5 mg/kg s.c.	164.1±47.1	272.1±71.6	0.6 ± 0.2	1.1±0.9
5 mg/kg s.c.	370.8±41	879.1±133.2	0.9 ± 0.6	0.9±0.1
10 mg/kg s.c.	893.9±90.7	2879.9±491.5	1.1 ± 0.4	2±0.6
Mouse				
3 mg/kg i.p.	369.8	---	0.17	0.6
10 mg/kg i.p.	1109±87	1233±53	≤0.3	0.4
20 mg/kg i.p.	2152±82	2611±86	≤0.3	0.6
Squirrel Monkey				
1.4 mg/kg p.o.	100.2±51.5	340.3±248.4	1±0.4	1.8±0.9
2.8 mg/kg p.o.	312.7±92.8	1314.2±581.5	1.1±0.4	2.1±0.8
5.7 mg/kg p.o.	723.6±228	3866.2±891	1.3±0.9	2.6±0.7
10 mg/kg p.o.	1594.5±295.6	12,839.2±2144.6	1.3±0.9	4.2±1.5
7.4 mg/kg s.c.	1227±167	5006±528	---	3.5±0.9
7.4 mg/kg p.o.	773±157	3408±821	---	3.1±0.5
Human				
1.0 mg/kg p.o.	147±10	1389±119	2.3±0.2	7.2±0.6
1.6 mg/kg p.o.	292±76	3485±760	2.4±0.6	8.1±2.1
1.6 mg/kg p.o.	254.7±60.4	3070.6±673.4	2.4±0.6	8.4±1.6

2.0 mg/kg p.o.	442-487	5133-5232	1.5-2.0	6.9-7.2

4.2.1 Absorption

At doses of 2 mg/kg, MDMA is rapidly absorbed by rats by both oral and subcutaneous routes of administration, with an observed Tmax of 0.56±0.31 hours by the oral route. At higher oral doses of 10 mg/kg a slightly shorter Tmax 0.31±0.13 hours were observed in rats.

At 2 mg/kg the p.o. to i.p. ratio of MDMA AUC can be calculated to be 0.37. This estimated relative bioavailability of 37% can be thought to approximate the absolute bioavailability of MDMA in rats. Note that at doses of 10 mg/kg in rats, the p.o. to i.p. relative bioavailability increases to 65%. At low doses, sex effects on exposure have been observed in rats. This effect dissipated at 5 mg/kg and is not expected nor observed in higher mammals or humans.

In squirrel monkeys, Mueller and colleagues observed an oral Tmax of 1 hour at 1.4 mg/kg and as long as 1.3 hours at 10 mg/kg. At doses of 7.4 mg/kg p.o. to i.p. relative bioavailability can be calculated of 68%.[53] Mechan and colleagues also observed increased exposure to MDMA upon repeated dosing, further illustrating MDMA's nonlinear pharmacokinetic characteristics. A 2011 article from Mueller and colleagues observed a prolonged Tmax of 7.1 hours at 5 mg/kg in baboons, a result not seen in other non-human primates[54].

4.2.2 Distribution

MDMA has been shown to be partially bound to plasma proteins in mammals. The mean unbound fraction of MDMA in plasma was measured in rabbits by De Letter and colleagues to be 63%±3 (n=6) at a concentration of 400 ng/ml[55]. In vitro plasma protein binding of MDMA in mice is lower than in rat and human serum, in which it is approximately equal[55]. This indicates that the same dose of MDMA in mice will have more active drugs available in serum than in rats and humans. Mice were shown to have lower plasma protein binding in vitro compared to rats and humans, and only mice exhibited stereoselective plasma protein binding.

The volume of distribution of MDMA at 1 mg/kg was observed to be 7.41 to 7.52 L/kg in rats. From Baumann and colleagues' published noncompartmental data, the volume of distribution is 4.43 to 9.52 L/kg in rats[44]. Work from De Letter and colleagues has identified a 2-compartment model of MDMA pharmacokinetics in rabbits. The total volume of distribution at a steady state was reported to be 4.9 L/kg. The model elucidated a central compartment of 1.9 L/kg, suggesting a large peripheral compartment in rabbits, leading to a biexponential half-life (alpha-$t_{1/2}$ of 5 minutes and a beta-$t_{1/2}$ of 63.5 minutes). The authors also observed some accumulation of MDMA in the vitreous fluid of rabbits.[55]

MDMA has been shown to have high distribution into the CNS compartment. Scheidweiler and colleagues observed a significantly higher AUC of MDMA in the striatum of mice across doses from 10 mg/kg to 40 mg/kg.[56] Striatum to plasma ratios were observed from 6.5 to 12.4, illustrating MDMA's high CNS penetration.

4.2.3 Metabolism

MDMA is metabolized via two hepatic pathways in rodents. In the major pathway in rats, MDMA is O-demethylated by cytochrome P450 CYP2D1 and 3A2 to form HHMA, which is O-methylated to generate HMMA by catechol-O-methyltransferase (COMT). In the minor pathway in rats, MDMA is N-demethylated by CYP1A2 and 2D1 to form MDA, which is an active metabolite. MDA is O-demethylated by the same enzymes as MDMA, with subsequent metabolism by COMT. Metabolites of MDMA are excreted in urine as glucuronide and sulphate conjugates. MDMA and metabolites have shorter half-lives in rats than humans at comparable doses based on plasma Cmax values. Rats tend to form more MDA and glucuronide-conjugated metabolites than humans. Figure 2 depicts the metabolic pathway of MDMA in animals and humans.

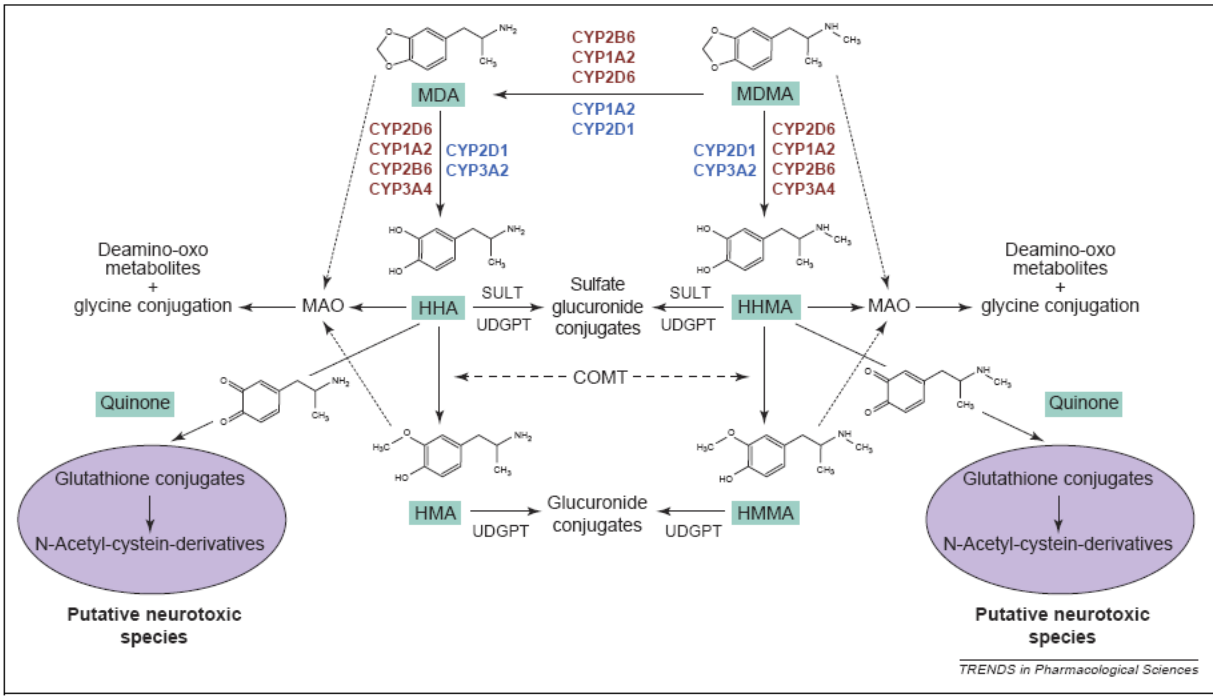

Figure 2: Metabolism of MDMA in human (red) compared to metabolism in rats (in blue).

As MDMA dose increases above 2.5 mg/kg subcutaneous (s.c.) or intraperitoneal (i.p.) in rats, a larger percentage of the administered dose is shunted to the N-demethylation pathway, resulting in greatly enhanced formation of MDA. Comparison of metabolic pathways between rats and mice given 10 mg/kg i.p. MDMA HCl indicates that 49.1% of MDMA is metabolized

through the HMMA pathway in mice versus 72% in rats, and 18.3% of MDMA is metabolized through the MDA pathway in mice versus 28% in rats based on AUC ratios to MDMA. MDMA HCl at 10 mg/kg was also found to be eliminated more rapidly in mice (0.4 hours, i.p.) than rats at (1.1 hours, s.c.)[15]

4.2.4 Elimination

Although hepatic metabolism is thought to be the major route of elimination across mammalian species, MDMA and its metabolites are present in the urine of rats for as long as 48 hours after a single 20 mg/kg MDMA HCl dose[15]. In contrast to most small molecule drugs, MDMA has been shown to have rapid clearance (the rate of drug elimination divided by plasma concentration) on the first day of life rats treated with 20 mg/kg of MDMA HCl. To date, there is no well-controlled animal study quantifying the excretion of MDMA into breast milk.

4.3 Toxicology

MDMA has been studied in single-dose and repeat-dose toxicology studies. MAPS has also published *in-vitro* and *in-vivo* safety studies addressing genotoxicity, fertility and early embryonic development, and embryo-fetal development with toxicokinetic analyses. Overall, MDMA appears to be well tolerated with no mortality or significant toxicologic findings after p.o administration to rats (<150mg/kg) and dogs(<9mg/kg) when single-housed [57].Summary of these studies are summarized in Table 1. For a detailed assessment of the findings, readers of this IB are encouraged to reference the full-text publication by MAPS [15].

4.3.1 Single Dose Toxicology Studies

Single doses between 5 and 100 mg/kg have been administered in rodents. Single doses in this range have transient effects on serotonin depletion, likely due to reversible inhibition of tryptophan hydroxylase, which prevents additional serotonin from being produced and released. A study on the long-term effects of a single dose of 5.7mg/kg MDMA HCl on estimated SERT sites in the brain of squirrel monkeys reported reduced SERT sites in some frontal, temporal and parietal areas [58] [59]. The maximum plasma concentration (Cmax) of 725 ug/L in squirrel monkeys was three times greater than what was observed in humans after a single oral dose of 100mg MDMA HCl.

MAPS has also sponsored a single-dose study of Sprague Dawley rats, where ten rats were exposed to 300mg/kg p.o.[60,61] Of these, 40% per sex died, and 12 survivors were euthanized 3 days later. Typical human doses being tested in the clinical trials are at least 100 to 150 times lower than the lethal dose tested in rodents, thereby suggesting relatively safe dosing. The oral single-dose LD50 was estimated, based on the aggregate mortality data from the study phases, to be 322 mg/kg[15].

4.3.2 Repeat-dose toxicology studies

Frith et al conducted a 28-day repeat-dose general toxicity study in both sexes of Sprague-Dawley rats (53 male, 52 female) (0, 10, 50, 100 mg/kg MDMA HCl oral gavage) and dogs (12 male, 12 female) (0, 3, 9, 15 mg/kg/day MDMA HCl oral dosing with gelatin capsules).[48] The initial highest dose was set at 18 mg/kg/day, but after the death of one female dog, the highest dose was subsequently reduced to 15 mg/kg/day. Dosing was once daily for 28 days. This research was performed within the U.S., a member of the Mutual Acceptance of Data (MAD) program, and studies were conducted in compliance with GLP based on available standards in 1986. Both sexes of dogs were administered 9 and 15 mg/kg/day of MDMA HCl, and rats receiving 50 and 100 mg/kg/day gained less weight than controls and the 3 mg/kg/day group, with significant differences in food consumption, observed as early as the first week which was no longer significantly different by the third week for the rats and the fourth week for the dogs.

Gross observations at autopsy in the dog possibly related to MDMA included reduced testicular size for one of three dogs receiving 9 mg/kg/day and one of three dogs receiving 15 mg/kg/day and prostatic enlargement in two dogs receiving 15 mg/kg/day. No gross lesions were seen in the rats at necropsy. Blood chemistry and urinalysis values were unremarkable in the dog. Clinical pathology findings showing a trend to decrease with dose in the rat were urinary pH, blood urea nitrogen, glucose, creatinine (females), lactate dehydrogenase (females), and chloride; in contrast, total white blood cell count (WBC) and phosphorus showed a trend to increase with dose. Histopathological examinations showed mild, diffuse atrophy in the two dogs with reduced testicular size. Mild focal atrophy was furthermore observed in the testes of one additional dog from the 15 mg/kg/day group. The two dogs with grossly enlarged prostates showed hyperplasia of the prostate. The silver stain for neuro histopathology was inconclusive in both the rat and the dog studies. These were interpreted as potentially MDMA-related effects as they were only seen in MDMA-dosed animals and not in vehicle-treated animals. In the rat, effects were observed in 5 of 10 male rats administered 100 mg/kg/day (none were observed in females). Although inconclusive, these potential effects were interpreted as vacuolated lesions apparent in the fiber tracts of the brainstem adjacent to the trigeminal nuclei. In the cerebrum of the dog, these effects included floccular changes of white matter, focal neural malacia and focal cellular infiltrates. Neural chromatolysis was observed in the brainstem. However, the authors noted that it is difficult to extrapolate the findings due to the low sample size (three dogs per sex per group).[15,48]

In a MAPS-sponsored pilot dose range-finding study of Sprague-Dawley rats, oral toxicity was assessed in a 13-day increasing dose regimen.[11] The initial dose started at 25 mg/kg and was increased by 25 mg/kg daily until 300 mg/kg was reached. Adverse reactions were observed in all doses above 25 mg/kg and included hyperexcitability, uncontrolled urination, piloerection, and bulging eyes. Tremors, muscle spasms, impaired movement, convulsions, and death were observed in the highest of dose levels, in the range of 150 and 300 mg/kg. Blood chemistry analyses suggested possible liver and kidney damage in animals receiving higher doses,

however, gross pathology and microscopy found no treatment-related damage to the liver. The only renal change observed via microscope was mild to minimal hydronephrosis, which the researchers attributed to treatment-related polyuria (abnormally large urine production). Minimal tubular atrophy was seen in the testes of 3 of the 20 treated male rats, suggesting a possible relationship between high dose, repeated MDMA treatment and minimal testicular atrophy in the male rat. Gross pathology revealed reddened lungs and enlarged urinary bladders (most likely polyuria-related) in some animals and were deemed potentially treatment-related. Histopathological observations of brain tissue revealed no signs of brain damage in any of the treated rats. [11]

MAPS also sponsored a 28-day repeat dose general toxicity study in both sexes of Sprague Dawley rats (10 male and 10 female per dose per arm) studying 0mg/kg to 25mg/kg of MDMA HCl p.o. Oral administration of 25mg/kg/dose of MDMA HCl was not tolerated in male rats, resulting in the mortality of six of twenty study males.

Clinical signs preceding euthanasia (n=1) included decreased activity, hunched posture, partially closed eyes and lateral recumbency. Clinical signs associated with oral administration of MDMA HCl (observed at 7.5mg/kg/dose and above, depending on the sign) varied by sex and included salivation, increased activity, red material around the nose, hypersensitivity to touch, hypersensitivity to sound, piloerection, stereotypy (head weaving), and aggression.[15]

Changes in FOB endpoints were consistent with the clinical signs. They included changes in endpoints related to activity/arousal, sensorimotor, neuromuscular, autonomic, and physiologic endpoints, most apparent 1-hour post-dose and trending towards resolution at 24 hours post-dose. Weekly oral MDMA administration resulted in decreases in mean body weight gain in animals administered 25/20 mg/kg/dose between Days -1 and 14 for males and Days -1 and 7 for females. This correlated with decreases in mean food consumption in males administered 25/20 mg/kg/dose between Days 7 and 14.

Changes in clinical pathology associated with MDMA administration included minimal decreases in mean urine volume and/or elevated urine specific gravity. They increased total protein, albumin and globulins, which was indicative of minimal subclinical dehydration in both sexes at 25/20 mg/kg/dose. Microscopically, MDMA-related changes were noted in the quadriceps, soleus and gastrocnemius skeletal muscles on Days 2 and 29. These findings included myofiber degeneration in the quadriceps (males ≥ 7.5 mg/kg/dose and females ≥ 2 mg/kg), soleus (25/20 mg/kg/dose males > females) and rarely in the gastrocnemius (25/20 mg/kg/dose males only) with infiltrations of mononuclear cells/leukocytes and/or neutrophils to varying degrees depending on muscle type (quadriceps>soleus> gastrocnemius). The basis for the difference was suspected to be related to a difference in myofiber type in the soleus muscles of rats.

At terminal necropsy within seven days after the last of four weekly repeated doses, myofiber degeneration was observed at a much lower incidence in the soleus muscles and rarely present

in the gastrocnemius muscle (males only). These observations may indicate some level of repair or adaption, as a low incidence of myofiber regeneration was also found at the MTD. Exposure of MDMA and active metabolite MDA as measured by Cmax and AUC(0-10hr) generally increased in a dose-proportional to greater than dose-proportional manner across the dose range with no notable differences in exposure related to sex and no evidence of accumulation or reduction in exposure after repeat administration. Taken together, weekly MDMA administration for 28 days (4 total doses) via oral gavage was tolerated in Sprague-Dawley rats up to 25 mg/kg/dose in females and 20 mg/kg/dose in males.

Due to the lack of any adverse findings or mortality after reducing the dose level in males, the NOAEL is determined to be 25 mg/kg/dose in females and 20 mg/kg/dose in males which correlated with Day 22 systemic exposures of 1490 ng/mL and 10700 hr*ng/mL (Cmax and AUC0- 10hr) in females and 1100 ng/mL and 4590 hr*ng/mL (Cmax and AUC0-10hr) in males. The risk of CNS neurotoxicity is minimal, however microscopic findings in the rat include a risk of neuromuscular inflammation in skeletal muscle in the periphery at human equivalent doses. An in vitro study reported MDMA activates nicotinic acetylcholine receptors (nAChR) at the neuromuscular junction, inducing calcium (Ca2+) transients and increasing acidification in myofibers, potentially leading to generalized denervation which precedes myofiber degeneration.

MAPS also sponsored a 28-day repeat dose study in beagle dogs (3 males, 3 females per dose per arm) and studied doses from 0 to 12 mg/kg MDMA HCl by oral gavage. Oral administration of 12mg/kg/dose of MDMA HCl was not tolerated, resulting in mortality of 4 dogs and one dog at 9 mg/kg/dose. Clinical signs preceding euthanasia included severe hypersensitivity to sound and touch, salivation, vocalization, aggression and (in the single animal administered 9 mg/kg/dose) unconsciousness. Clinical signs associated with oral MDMA were most prominent on Day 1 (generally resolving within 24 hours of dosing), diminished at subsequent dosing intervals and included increased salivation, injected sclera, hypersensitivity to touch and vomitus/emesis/retching at ≥ 2 mg/kg/week; lacrimation/ocular discharge (yellow or green; which was observed in a single 12 mg/kg animal during ophthalmoscopic exams on Day 2), and increased activity at ≥ 4 mg/kg/week; and dilated pupils, aggressive behavior, hypersensitivity to sound, vocalization, trembling, partially/completely closed eyelids, stereotypy/abnormal head movements (jerking and side to side), audible breathing, coughing and cold skin at 12/9 mg/kg/week. Changes in the FOBs were consistent with the clinical signs and included endpoints related to activity/arousal, sensorimotor, neuromuscular, and autonomic endpoints that are consistent with clinical observations described above. Changes were generally similar between the sexes and were most apparent at 1-hour post-dose on Day 1, with a majority of changes trending towards resolution by 24 hours post-dose. Most of the changes in FOB endpoints observed on Day 1 were also observed on Day 22. Oral MDMA administration resulted in a reduction in mean body weight during Week 1 and an overall decrease in mean body weight gain through the dosing phase in males administered 12/9 mg/kg/week. Post-dose decreases in food consumption were observed at all dose levels (including controls) predominantly during Week 1. However, this decrease in food consumption

did not translate into a body weight effect in any dose level except 12/9 mg/kg males. Following weekly oral gavage administration of MDMA, exposure (mean Cmax and AUC0- 8hr) of MDMA and MDA increased with increasing dose in an approximately dose-proportional manner from 2 to 9 mg/kg, with no apparent increase from 9 to 12 mg/kg (N=1) on Day 1 and increased with increasing dose in an approximately dose-proportional manner from 2 to 9 mg/kg on Day 22 with no evidence of accumulation or differences in sex. Systemic exposure (AUC0- 8hr) to MDA appeared similar to the systemic exposure of MDMA on Days 1 and 22. Taken together, weekly oral administration of MDMA for 4 weeks (4 total doses) was tolerated in dogs up to 4 mg/kg/week. Due to the mortality/early termination at 12 and 9 mg/kg/week, the NOAEL for this study is determined to be 4 mg/kg/week which correlated with peak and cumulative exposures of 219 ng/mL and 139 ng/mL (Cmax) and 983 hr*ng/mL and 897 hr*ng/mL (AUC0- 8hr) for MDMA and MDA, respectively.

4.3.3 Genotoxicity

Genotoxicity studies conducted by Charles River Laboratory supported the conclusion that MDMA is :

- negative for mutagenic activity in salmonella typhimurium and Escherichia coli
- negative for genotoxicity activity in CHO cells in the presence and absence of metabolic activation
- negative for clastogenic activity and/or disruption of the mitotic apparatus under the assay conditions.

Further based on genotoxicity studies, there is no cause for concern of carcinogenic risk of MDMA. No tumors were reported after daily 28-day repeated dose toxicology studies of MDMA in rats or dogs described above.

4.3.4 Reproductive and Developmental Toxicity

Preliminary teratological studies in rats (N=12 per dose) given 0, 2.5, or 10 mg/kg MDMA HCl (with correction factor for HCl salts) by gavage on alternate gestational days (GD) 6 to 18 found no abnormalities in gestational duration, litter size, neonatal birth weights, or birth defects (N=10 litters per dose), despite statistically significant reduction in maternal weight gain at 10 mg/kg [62].

MAPS has completed a definitive GLP study of the effects of MDMA on fertility and early embryonic development to implantation in both sexes of rats. MDMA-related decreases in body weight, weight gain, and food consumption were expected pharmacological effects based on repeat-dose studies. The NOAEL was demonstrated to be the highest dose level evaluated ≤10 mg/kg/day (supratherapeutic) in both sexes for fertility and reproductive performance[15]. Complete data sets from the MAPS-sponsored GLP toxicology studies can be found in the MAPS investigator brochure publicly available on the MAPS website.

MAPS has also completed a definitive GLP embryo/fetal developmental toxicology study in time- mated female New Zealand White Hra:(NZW) SPF rabbits. Rabbits were dosed on GD7-19 at 0 mg/kg/day (vehicle control), 2.5 mg/kg/day, 5 mg/kg/day, and 10 mg/kg/day MDMA

(with correction factor for HCl salt) by gavage (N=22 each). MDMA was well-tolerated at dose levels ≤10 mg/kg/day. Following daily oral gavage administration of MDMA to pregnant rabbits, Cmax and AUC0-24hr values for MDA increased with increasing dose in a greater than dose proportional manner on GD 7 and GD 19. Systemic exposure (AUC0-24hr values) to MDA increased following repeated administration of 2.5, 5, and 10 mg/kg MDMA HCl to pregnant rabbits. Systemic exposure (AUC0-24hr values) to MDA was 1 to 3 times greater than the systemic exposure to MDMA on GD 7 and GD 19. Based on the results of this study, the NOAEL for maternal and developmental toxicity was considered to be 10 mg/kg/day, the highest dose level evaluated, with a maternal MDMA Cmax of 603 ng/mL and AUC0-24hr of 2920 hr*ng/mL on GD 19. [15]

4.3.5 Other Toxicity studies

Immunological effects

MDMA may act as a mild immunosuppressant in rodents, with no evidence of immunotoxicity in repeat-dose toxicology studies, as demonstrated by MAPS [15]. However, it is worth stating that a trend toward a dose-dependent increase in white blood cells was noted in the dog study, which could be related to an increase in cortisol secretion induced by MDMA. Single-dose MDMA administration in rats at 5mg/kg is associated with impaired macrophage activity as evidenced by inhibition of tumour necrosis factor-alpha (TNF-alpha) secretion for 12 hours post-drug. In mice injected with 10 mg/kg MDMA HCl for 5 days, increases in epithelial tissue of cytokines interleukin 1-alpha (IL-1alpha), granulocyte-colony stimulating factor (G-CSF), and interleukin 3 (IL-3) were found, while decreased serum levels of many cytokines were reported. MDMA decreased neutrophil oxidative bursts and phagocytosis and increased the number of circulating neutrophils while decreasing the number of lymphocytes. Incubating photoreceptor-generated cells with 0.5, 1 and 2 μM, MDMA HCl activated macrophages led them to release proinflammatory cytokines (42). MDMA also increased hypothalamus-pituitary-adrenal (HPA) axis activity through a noradrenergic pathway in the hypothalamus[33]. To the best of our knowledge, there have been no publications reporting an increased occurrence of tumours or infections associated with MDMA administration to date.

Mechanistic studies

A series of studies examining neurogenesis, a marker of neuroplasticity, found that MDMA and classic psychedelics stimulated neurite growth, with this BDNF-dependent effect blocked by 5HT2A antagonists. MDMA was more effective than ketamine in promoting neurite growth. These findings may lie behind some of the therapeutic effects of MDMA, such as enhanced fear extinction learning, greater sensitization to prosocial effects and or re-learning or re-opening experiencing social reward. Still, behavioural effects were not specifically tested in this report. [15]

Several research teams have studied the effects of MDMA on gene expression in rodents. Many of these reports used 10 to 20 mg/kg MDMA HCl. Toxicity was not observed at these

doses, and effects broadly indicated changes in memory and cognition. Particularly of interest for treatment of PTSD, one study found downregulation of the gene for several glutamate receptor genes, several calcium transport genes, and the cannabinoid receptor CB1, among other effects. Another study found that MDMA influenced genes of proteins known to regulate glutamatergic signaling and are associated with neuroplasticity and learning, as well as processes involved in memory consolidation in serotonergic neurons. These studies also report an increase in the expression of genes that regulate the GABA transporter, which is expressed in GABAergic neurons indirectly regulated by glutamatergic afferent neurons. Serotonin-transporter knockout mice did not display some of these changes in gene transcription, suggesting that serotonin release is required for this activity. In the acute period 24 to 48 hours after MDMA exposure, a study in rats found 33 to 70% upregulation of BDNF messenger ribonucleic acid (mRNA) transcripts in the frontal cortex, with a time-dependent decrease, up to 73%, of BDNF transcripts in the hippocampus. The frontal cortex and hippocampus are both regions known to play a causal role in memory retrieval and reconsolidation in animals and humans, mediated in part through GABAergic signalling. Changes in transcription do not always correlate with functional consequences in proteins levels.
BDNF has been shown to have multiple functionally distinct splice variants which have tight temporal and spatial control in an activity-dependent, stimulus-specific manner. However, MDMA produces a durable enhancement of fear extinction in mice, an effect mediated by an MDMA-associated increase in BDNF expression specifically in the context of fear extinction training, supporting that gene expression changes after MDMA are functionally relevant. [15]

Abuse potential

Several studies have investigated the abuse liability of MDMA in animals through paradigms of drug seeking, drug discrimination, and withdrawal. Mice, rats, and monkeys self-administer MDMA, indicating that MDMA has rewarding properties in animals; however, the rate and response-acquisition of self-administration are much lower than other drugs of abuse, such as cocaine or heroin. [63–67]

Rodent studies found that training attempts at self-administration required an increased training dose of 1.75 mg/kg for acquisition over five weeks[63,68]. Research that used the ability of a drug to impact the rate of intracranial self-stimulation (ICSS) as a measure of abuse liability compared the impact of 0.32, 1, or 3.2 mg/kg MDMA HCl pre-treatment in male and female rats. At the 3.2 mg/kg dose, MDMA increased responding for ICSS when the rate of responding for ICSS was low and reduced seeking ICSS when the rate of responding was very high in both sexes.

Physical dependence and drug withdrawal were investigated by treating rats with 10 mg/kg i.p. MDMA HCl twice daily for five days. When compared with rats trained to self-administer cocaine, MDMA-trained rats were less likely to return to self-administration after a period of abstinence. Monkeys choose to self-administer MDMA in doses equivalent to or only slightly higher than doses used by humans [15].

Discrimination research in a sample of monkeys trained to discriminate cocaine from saline and tested with cathinones, amphetamines, and MDMA and MDA, suggested that the greater serotonergic effects of MDMA are at least partially related to the methylenedioxy structure[69,70]

5.0 Effects in humans in clinical settings

The first documented use of MDMA in humans' dates back to the early 1970s by Shulgin and Nichols. Legal therapeutic use of MDMA continued until its placement on the US list of Scheduled I drugs in 1985. Controlled human studies of MDMA commenced in the mid-1990s with a MAPS-funded investigator-initiated Phase 1 dose-response safety study [15]. As of February 29, 2024, clinicaltrials.gov reports 145 clinical trials have been registered, of which 25 have not yet started recruiting, 79 studies have been completed with 20 trials where the results have been published, and five where either the trial was terminated, withdrawn, or suspended.

MAPS has concluded twenty-one sponsor-supported studies of MDMA-assisted therapy for PTSD. Table 3 below gives an overview of the studies completed thus far, including the healthy human volunteer studies, and the safety of MDMA-assisted therapy for anxiety and social anxiety.

Table 3: Summary of MAPS sponsored Trails with MDMA

NCT #	Location	Population	MDMA HCl Initial Dose[B]	MDMA HCl Supplementary Dose[B,C]	Design
NCT00090064 (MP-1)	USA	Crime, veterans	Placebo (n=8),	Placebo,	Blinded RCT, open-label Stage 2
			125 mg (n=15)	62.5 mg	
NCT00353938 (MP-2)	EU	Various	25 mg (n=5),	12.5 mg,	Blinded RCT, open-label Stage 2
			125 mg (n=9)	62.5 mg	Open label Stage 3
NCT00402298 (MP-3)	Israel	Various	25 mg (n=2),	12.5 mg,	Blinded RCT, planned Stage 2
			125 mg (n=3)	62.5 mg	
NCT01958593 (MP-4)	Canada	Various	Placebo (n=2),	Placebo,	Blinded RCT, open-label Stage 2
			125 mg (n=4)	62.5 mg	
NCT01211405 (MP-8)	USA	Veterans, firefighters, police officers	30 mg (n=7),	15 mg,	Blinded RCT, open-label Stage 2
			75 mg (n=7),	37.5 mg,	
			125 mg (n=12)	62.5 mg	
NCT01689740 (MP-9)	Israel	Various	25 mg (n=3),	12.5 mg,	Blinded RCT, open-label Stage 2
			125 mg (n=7) E	62.5 mg	
NCT01793610 (MP-12)	USA	Various	40 mg (n=6),	20 mg,	Blinded RCT, Open label Stage 2
			100 mg (n=9),	50 mg,	
			125 mg (n=13)	62.5 mg	
NCT02876172 (MPVA-1)	USA	Dyads w/one	75 mg (1st session),	37.5 mg,	

NCT #	Location	Population	MDMA HCl Initial Dose[B]	MDMA HCl Supplementary Dose[B,C]	Design
		person w/ PTSD diag., one no PTSD	100 mg (2nd session)	50 mg	Open label Phase 1/ Phase 2
NCT03282123 (MP-16)	Multi-site; US, Israel	Various	80 mg,	40 mg,	Open label
			100 mg	50 mg	
NCT03485287 (MP-17)	Multi-site, Canada	Various	100 mg,	50 mg,	Open label
			125 mg	62.5 mg	
NCT03537014 (MAPP1)	Multi-site; US, Canada, Israel	Patients with atleast severe PTSD	Placebo	Placebo,	Phase 3
			80 mg (1st sessions), 80 or 120 mg (2nd, 3rd sessions)	40 mg,	
				40 mg or 60 mg	
NCT02008396 MAA-1	USA	Adults on autism spectrum with social anxiety	Placebo (n=4),	Placebo,	Blinded RCT, open-label arm
			75-125 mg (8)	32 mg to 53 mg	
NCT02427568 MDA-1	USA	Anxiety related to life-threatening illness	Placebo (n=5),	Placebo,	Blinded RCT, Open Label Stage 2
			125 mg (n=13)	62.5 mg	
NCT03181763 MPVA-4	USA	Healthy, aged 21-55, prev. experience w/MDMA	Placebo,	Placebo,	Between-subjects RCT
			100 mg	50 mg	
NCT01458327 (MPV1-E2)	USA	People Who Relapsed After Participating in a Phase 2 Clinical Trial	106 mg	53 mg	Phase 2
NCT01404754	USA	Healthy	106 mg	53 mg	Phase 1

NCT #	Location	Population	MDMA HCl Initial Dose[B]	MDMA HCl Supplementary Dose[B,C]	Design
(MT-1)		volunteers over the age of 18			
NCT04784143 (MPVA-6)	USA	Persons with moderate PTSD over age of 18	100 mg	34 mg	Phase 2
NCT04030169 (MP18)	Europe	Persons with non-dissociative subtype of PTSD	100 mg	34 mg	Phase 2
NCT05147402 (MPKF)	USA	Healthy persons	100 mg	n/a	Phase 1
NCT04714359 (MAPPUSX)	USA, Israel, Canada	Persons with at least moderate post traumatic stress disorder over the age of 18	100 mg	34 mg	Phase 3
NCT04077437 (MAPP2)	USA, Israel	Patients with atleast severe PTSD	Placebo	Placebo,	Phase 3
			80 mg (1st sessions), 80 or 120 mg (2nd, 3rd sessions)	40 mg,	
				40 mg or 60 mg	

Abbreviations: NCT=clinicaltrials.gov identifier; n=number of participants; m=months; w=weeks; TBD=To be determined

A Completed Studies indicates that all subjects have completed the study.

B Values reflect the active MDMA free-base weight, not the total MDMA HCl weight.

C Study protocols include a divided-dosing regimen with an initial dose followed by a supplemental dose administered 1.5-2 hours later, unless tolerability issues emerge with the initial dose or the participant declined.
D At treatment exit.
E The first two participants were open-label (125 mg MDMA) and were included in the efficacy analyses

5.1. Phase 1 Studies

MAPS has one completed (NCT03181763) Phase 1 study. NCT03181763, a Phase 1, randomized, placebo-controlled, double-blinded between-groups study in 34 healthy participants examining the effects of MDMA on the presence and intensity of startle response after receiving cues previously paired with a startling stimulus. Participants received 100 mg MDMA HCl or placebo and repeated the startle-related task with the same cues in the absence of the startling stimuli to assess the effect of MDMA or placebo on the startle response. The results of the study published in March 2022 demonstrated MDMA was well tolerated amongst the clinical trial participants, although the authors report they did not observe the hypothesized facilitation of extinction retention.[71]

Study MPFK was a Phase 1 (MAPS-sponsored) study that evaluated the effect of a single dose of 120mg of MDMA in 16 healthy volunteers in the fed and fasted state. Blood pressure and heart rate were monitored up to 72 hours after dosing. Electrocardiograms were also collected in this study. Results demonstrated that there was no effect of food on Cmax and AUC of MDMA or MDA (metabolite). There was, however, a noted delay in Tmax when MDMA was administered in the fed state with a high-fat/high-calorie meal (Table 4) , which did not generally affect the time course analysis of subjective effects. No participants developed QT prolongation or experienced TEAEs suggestive of ventricular arrhythmia. [72]

Table 4 Plasma PK parameters of MDMA following a single dose 120mg Oral dose of MDMA HCl under fasting or fed conditions (Study MPKF) Ref: 73,74

Parameter[a]	units	Fasted (N=15) Geometric Mean (Geo CV %)		Fed (N=11) Geometric Mean (Geo CV %)	
AUC_{0-t}	h*ng/mL	3123	(42.5)	3060	(46.2)
AUC_{0-72}	h*ng/mL	3368	(37.9)	3301	(42.7)
AUC_{0-inf}	h*ng/mL	3388	(38.4)	3318	(43.3)
C_{max}	ng/mL	238	(24.7)	227	(21.7)
T_{max}[b]	h	2	(2.00, 8.00)	4	(4.00, 6.00)
$t_{1/2}$	h	8.7	(21.8)	8	(27.2)
T_{lag}[b]	h	0.6	(31.3)	0.7	(38.4)
CL/F	L/h	29.5	(38.4)	30.1	(43.3)

Vd/F	L	371.7	(23.5)	349.4	(22.6)

a: Arithmetic mean (Arithmetic CV%); N
b: Median (Min, Max); N
AUC_{0-t} = Area Under the Plasma Concentration-Time Curve from Time 0 to the Time of the Last Measurable Concentration;
AUC_{0-72} = Area Under the Plasma Concentration-Time Curve from Time 0 to 72;
AUC_{0-inf} = Area Under the Plasma Concentration-Time Curve from Time 0 to Infinity;
C_{max} = Maximum Observed; Plasma Concentration;
T_{max} = Time of Observed Maximum Plasma Concentration;
$T_{1/2}$ = Apparent Terminal Elimination Half-Life;
T_{lag} = Delay in Achieving T_{max};
CL/F = Apparent Total Plasma;
Clearance; V_d/F = Apparent Volume of Distribution;
h = Hours; N = Number of Participants in the Analysis Population.

Bershad et al. examined the effect of MDMA, compared to placebo and methamphetamine, on responses to positive and negative social feedback in healthy volunteers in a double-blind, placebo-controlled, cross-over trial (NCT03790618).[75] 36 healthy volunteers aged 18-40 years, with a BMI between 19 and 30 kg/m^2 ingested a capsule containing methamphetamine (20mg), MDMA (0.75 or 1.5mg/kg), or placebo. Following administration, participants relaxed for an hour and subjective and cardiovascular measures were collected at varying time points throughout the day and at the end of the session.
It is not surprising to note that both MDMA (both doses) and methamphetamine increased ratings of "feel drug," "like a drug," "feel high," and "want more." However, at 1.5mg/kg MDMA, the score for "dislike" drug was the highest and significantly more than placebo.[75]

Straumann, I. et al. in another Phase 1 study, used a double-blind, randomized, placebo-controlled, crossover design to compare the acute effects of racemic MDMA (125 mg), S-MDMA (125 mg), R-MDMA (125 mg and 250 mg), and placebo in 24 healthy participants.[76] Their outcome measures included subjective, autonomic, adverse effects, pharmacokinetics, plasma oxytocin, cortisol and prolactin concentrations. S-MDMA produced overall greater subjective effects than MDMA and R-MDMA at the doses tested (Figure 3). Responses in female participants were greater than in male participants, probably due to lower body weights in women. Interestingly, responses in participants with and without prior MDMA experiences also did not differ[76].

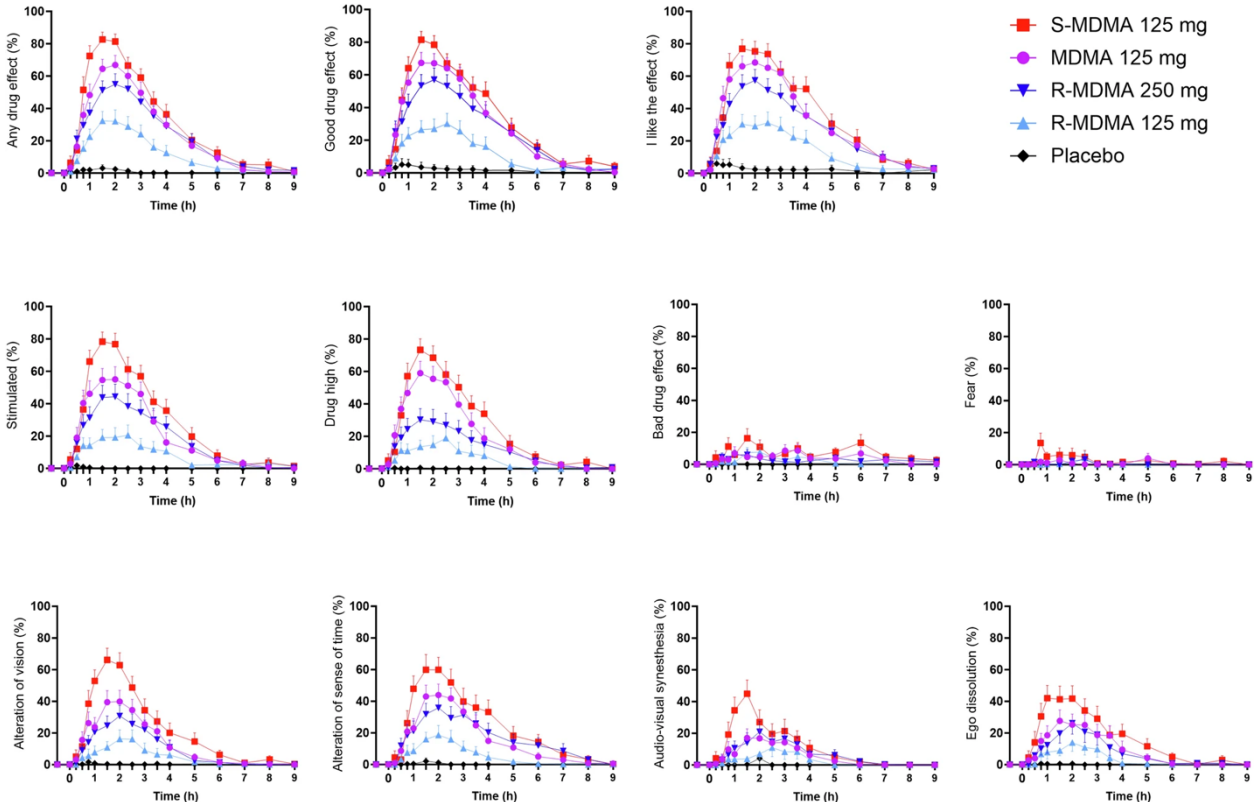

Figure 3 Acute subjective effects of 125 mg MDMA, 125 mg S-MDMA, 125 mg R-MDMA, and 250 mg R-MDMA on the Visual Analog Scale (VAS)

S-MDMA induced greater increases in blood pressure compared with MDMA and both R-MDMA doses. MDMA, S-MDMA and 250mg R-MDMA increased heart rate and body temperature comparably (Figure 4). All substances produced similar acute and subacute adverse effects. Frequently reported adverse effects included fatigue, headache, decreased appetite, feeling dull, lack of concentration, and dry mouth. All substances nominally increased self-ratings of depressive mood on the BDI 1–3 days after substance administration. Significantly higher ratings were seen for S-MDMA compared with placebo, with no significant differences between active drug substances.

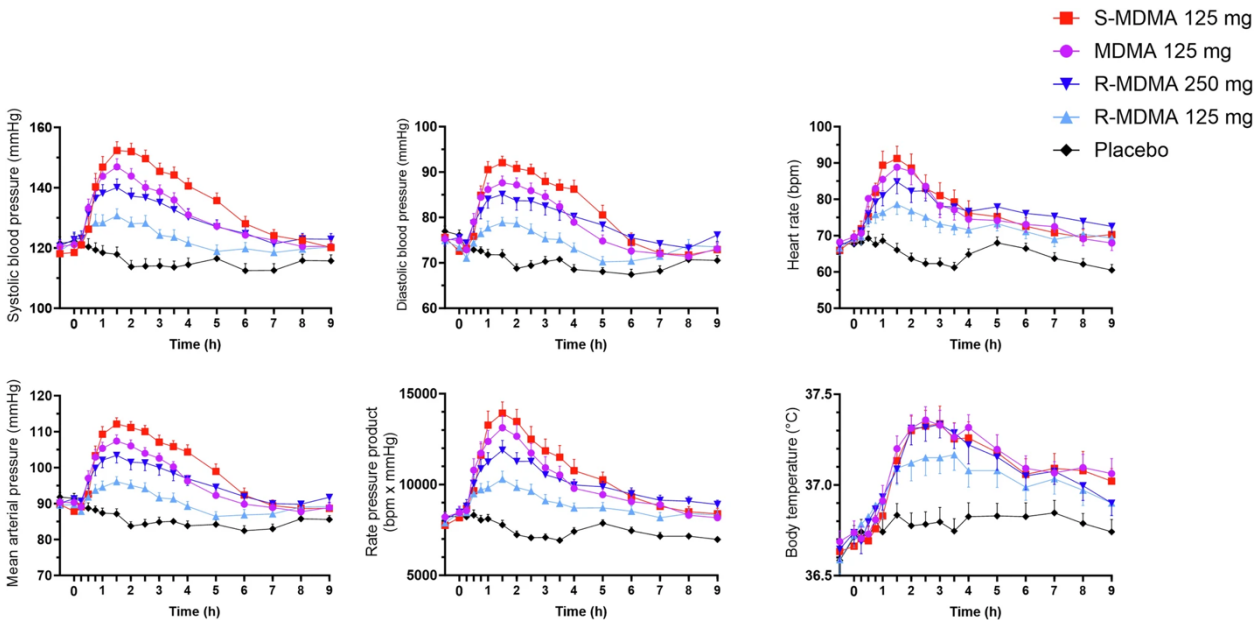

Figure 4 Acute autonomic effects of R-MDMA, S-MDMA, MDMA and placebo in healthy human volunteers

All substances increased plasma prolactin and cortisol compared with placebo. S-MDMA increased plasma prolactin more than MDMA and plasma oxytocin and cortisol more than MDMA and R-MDMA (Figure 5) [76] .

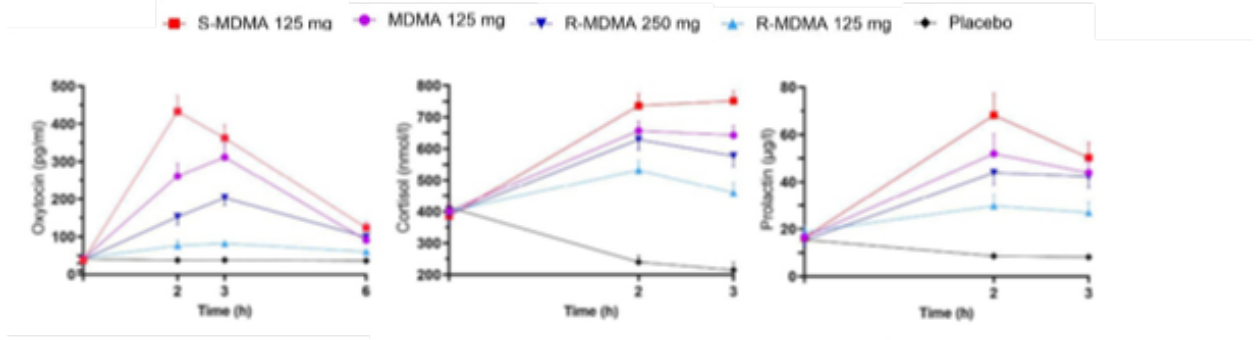

Figure 5 Plasma concentrations of oxytocin, cortisol, and prolactin with MDMA, S-MDMA.

5.2. Phase 2 Studies

Most data reported are from Phase 2 studies of MDMA-assisted therapy for PTSD. Data from a pair of investigations of MDMA-assisted therapy for other indications are also presented. These studies have administered MDMA in divided doses, with an initial dose administered at the start of the session and a supplemental dose following 1.5 to 2 hours later. The supplemental dose given at approximately peak exposure from the initial dose does not significantly impact the intensity of pharmacodynamic effects but does enable a longer period to process trauma in the context of dosing sessions. Studies have employed a range of comparator and active doses from an initial dose of 25 mg to 150 mg MDMA HCl. The lowest dose in use has been 25 mg MDMA HCl, used as a placebo or low dose comparator. Typically, Stage 1 was blinded, and Stage 2 was open-label in which participants who received placebo or comparator doses in

Stage 1 received active MDMA. The highest initial dose (150 mg MDMA HCl) was offered to a limited number of participants in MP-2 as part of "Stage 3," an open-label arm for non-responders in Stage 1 and/or Stage 2. The most common dosing regimen employed was 125 mg MDMA HCl followed by a supplemental dose of 62.5 mg as the primary active treatment. The supplemental dose given at approximately peak exposure from the initial dose does not significantly impact the intensity of pharmacodynamic effects but does enable a longer period to process trauma in the context of dosing sessions. In MAPS-supported studies, MDMA or placebo/comparator is administered after preparatory therapy during two or three 8-hour dosing sessions scheduled 2 to 5 weeks apart, each followed by at least three sessions of integrative therapy.

MAPS has completed eight blinded, randomized, controlled and four open-label Phase 2 and Phase 3 investigations of MDMA-assisted therapy for PTSD, including one extension study for the treatment of relapse[17]. These studies explored the reproducibility and persistence of treatment outcomes of MDMA-assisted therapy in people with chronic PTSD and, in some protocols, PTSD that failed to respond to at least one course of treatment or at least one course of pharmacotherapy. Mithoefer et al. evaluated these phase 2 trials in a pooled analysis. After two blinded experimental sessions, the authors noted that the active group had significantly more significant reductions in CAPS-IV total scores from baseline than the control group [MMRM estimated mean difference (SE) between groups – 22.0 (5.17), $p<0.001$]. After two experimental sessions, more participants in the active group (54.2%) did not meet CAPS-IV PTSD diagnostic criteria than the control group (22.6%). Depression symptom improvement on the BDI-II was greatest for the active group compared to the control group, although only trended towards significant group differences [MMRM, estimated mean difference (SE) between groups − 6.0 (3.03), $P = 0.053$]. All doses of MDMA were well tolerated, with some expected reactions occurring at a greater frequency for the active MDMA group during experimental sessions and the seven days following. Treatment-emergent adverse events during the blinded treatment segment and expected reactions during two blinded MDMA sessions are captured in Table 5 below. These studies demonstrated that MDMA-assisted psychotherapy was efficacious and well-tolerated in a large sample of adults with PTSD.

Table 5: Treatment emergent adverse events (Phase 2 MDMA-assisted psychotherapy)

	Control ($n = 31$)	Active MDMA ($n = 72$)	Total ($n = 103$)
Top reactions during experimental sessions, *n* (%)[a]			
Anxiety	15 (48.39)	52 (72.22)	67 (65.05)
Dizziness	6 (19.35)	29 (40.28)	35 (34.00)
Fatigue	18 (58.06)	35 (48.61)	53 (51.46)
Headache	22 (70.97)	38 (52.78)	60 (58.25)
Jaw clenching, tight jaw	6 (19.35)	46 (63.89)	52 (50.49)
Lack of appetite	7 (22.58)	35 (48.61)	42 (40.78)
Nausea	6 (19.35)	29 (40.28)	35 (33.98)
Psychiatric TEAEs, *n* (%)[b]			
Anxiety	3 (9.7)	17 (23.6)	20 (19.4)
Depressed mood	1 (3.2)	6 (8.3)	7 (6.8)
Irritability	0	3 (5.6)	3 (2.9)
Panic attack	0	3 (5.6)	3 (2.9)

TEAE, treatment-emergent adverse event

[a] Frequency of subjects who reported an expected, spontaneously reported reaction collected during blinded experimental sessions 1 and 2 (only reactions reported by ≥ 40% of participants in any group are displayed; see supplemental for full list of reactions)

[b] Frequency of subjects who self-reported psychiatric adverse events after first drug administration until the day before experimental session 3 (only AEs reported by three or more subjects in either group displayed)

MP-8 [72] was a randomized, triple-blinded, dose-response, Phase 2 study (sponsored by MAPS) to assess the safety and efficacy of MDMA in veterans, firefighters, and police officers diagnosed with chronic, treatment-resistant PTSD. Participants were randomized 1:1:2 to receive a total split dose of 45 mg (30 + 15 mg; low dose), 112.5 mg (75 + 37.5 mg; medium dose), or 187.5 mg (125 + 62.5 mg; high dose) MDMA. The primary efficacy endpoint was in PTSD symptoms by Clinician-Administered PTSD Scale for DSM-IV (CAPS-IV) global scores from baseline to 1 month after medication session-2. Safety was assessed by AEs, SRRs, C-SSRS, vital signs, subjective units of distress (SUD), and visual analog scale (VAS) (pre-existing tinnitus, chronic pain). A total of 26 participants were enrolled and randomized to receive [72] either 45 mg (low dose; n = 7), 112.5 mg (medium dose; n = 7), or 187.5 mg (high dose; n = 12) MDMA during each medication session. Reductions in CAPS-IV score at the Stage 1 Primary Endpoint compared to baseline were observed overall and in each dose group, and the CAPS-IV difference score from baseline in the low dose group was significantly different from the difference score in the medium (p = 0.002) and high (p = 0.022) dose groups. MDMA was generally well tolerated. No deaths were reported during this study. There were no TEAEs that led to premature study withdrawal.

MP-12 [72] (sponsored by MAPS) was a Phase 2 randomized, double-blinded dose-ranging study examining the safety and efficacy of MDMA in participants with chronic, treatment-resistant

PTSD of at least 6 months duration. Participants were randomized 9:9:5 to receive a total split dose of MDMA dose 1 of 187.5 mg (125 + 62.5 mg), dose 2 of 150 mg (100 + 50 mg), or low dose of 60 mg (40 + 20 mg). A split dose format was used, with the second part of the dose administered 1.5 to 2.5 hours after the first part. The second part of the split dose could be declined by the participant or withheld at the discretion of the clinical investigators. A total of 28 participants were enrolled and randomized to either the dose 1 group (N = 13), dose 2 group (N = 9), or low dose group (N = 6). All participants completed study termination visits.

The MP-12 study consisted of Stage 1 with two blinded (medication session 1; MS-1, medication session 2; MS-2) and one open-label (medication session 3; MS-3) dosing MDMA, and an End-of-Stage 1 follow-up; and a Stage 2 with three open-label medication sessions (MS4, MS5, and MS6) and a 12-month long term follow-up after the last medication session. In Stage 1, participants received either MDMA dose 1 of 187.5 mg (125 + 62.5 mg MDMA), MDMA dose 2 of 150 mg (100 + 50 mg MDMA), or a low dose of 60 mg (40 + 20 mg MDMA). Participants who received the low dose MDMA [40 mg]) in Stage 1 had the opportunity to crossover into Stage 2 where they received 3 OL MSs 150 mg (100 + 50 mg MDMA) during the first session and 150 mg (100 + 50 mg MDMA) or 187.5 mg (125 + 62.5 mg MDMA) in the second or third sessions.

A reduction in global CAPS-IV total severity score at the Stage 1 primary endpoint was observed in participants treated with MDMA in the study. At the Stage 1 primary endpoint, 19 (70.4%) participants had a clinically meaningful 15-point reduction in global CAPS-IV total severity score and 11 (40.7%) participants no longer met the PTSD Diagnostic Criteria. There was a greater reduction in global CAPS-IV score from the MDMA 187.5 mg and MDMA 150 mg dose treatment groups compared to the low dose treatment groups after 2 ESs. At long-term follow-up, the mean CAPS-IV scores of all treated participants were significantly reduced from baseline. MDMA was generally well tolerated. No deaths were reported during the study.

MAPS has also completed one Phase 2, placebo-controlled, double-blinded study of MDMA-assisted therapy in autistic adults with social anxiety (n=12), which demonstrated improvement in social anxiety symptoms in the study population[77]. Autistic adults with marked to very severe social anxiety were randomized to receive MDMA (75 to 125 mg, n = 8) or inactive placebo (0 mg, n = 4) during two 8-h psychotherapy sessions (experimental sessions) in a controlled clinical setting. Double-blinded experimental sessions were spaced approximately 1 month apart with 3 non-drug psychotherapy sessions following each. The primary outcome was a change in Leibowitz Social Anxiety Scale (LSAS) Total scores from Baseline to one month after the second experimental session. Outcomes were measured again six months after the last experimental session. Improvement in LSAS scores from baseline to the primary endpoint was significantly greater for the MDMA group compared to the placebo group (P = 0.037), and placebo-subtracted Cohen's d effect size was very large (d = 1.4, CI − 0.074, 2.874). Changes in LSAS scores from baseline to 6-month follow-up showed similar positive results (P = 0.036),

with a Cohen's d effect size of 1.1 (CI – 0.307, 2.527). Social anxiety remained the same or continued to improve slightly for most participants in the MDMA group after completing the active treatment phase. This Phase 2 trial thus demonstrated rapid and durable improvement in social anxiety symptoms in autistic adults following MDMA-assisted psychotherapy. Table 6 discusses the treatment-emergent adverse events. Initial safety and efficacy outcomes support the expansion of research into larger samples to investigate this novel treatment for social anxiety further.

Table 6: Number of participants reporting Treatment-emergent psychiatric adverse events in the Phase 2 Clinical trial with autistic adults [57]

	Placebo (*n* = 4)	MDMA (*n* = 8)
Psychiatric TEAEs, no. (%)[a]		
Anxiety	0	1 (12.5)[b]
Depressed mood	0	2 (25.0)
Depression	1 (25.0)	1 (12.5)[c]
Panic attack	0	1 (12.5)[b]
Panic reaction	0	1 (12.5)
Suicidal ideation	1 (25.0)	2 (25.0)[b]
None	3 (75.0)	4 (50.0)

Abbreviations: TEAEs, treatment emergent adverse events
[a]Frequency of subjects who self-reported psychiatric adverse events after first drug administration until the primary endpoint
[b]One moderate
[c]Two moderate

MAPS has also completed one study of MDMA-assisted therapy in people experiencing anxiety in the face of a life-threatening illness (MDA-1, N=18), which demonstrated that MDMA was well tolerated in the population. Still, differences in the state-trait anxiety scale did not reach statistical significance despite a large effect size. [78]

5.3. Phase 3 Study

MAPP1 and MAPP2 pivotal Phase 3 trials were conducted to support planned marketing authorization applications for approval of MDMA-assisted therapy to treat PTSD.
MAPP1 enrolled 131, randomized 91, and treated 90 participants who received receive MDMA (N=46) or placebo (N=44) with identical therapy. MAPP2 enrolled 121, randomized 104, and treated 103 participants who received receive MDMA (N=53) or placebo (N=50) with identical

therapy[9]. The 120 mg (80mg + 40mg) split dose was selected for the first experimental session in phase 3 trials to allow patients to acclimate to the treatment regiment using a clinical titration approach based on clinical recommendations from a phase 2 trial in veterans and first responders [9]. The supplemental dose and dose escalation could be withheld if the initial dose was not tolerated or the participant declined.
MAPP1 began enrollment in November 2018 and completed the study in October 2020. MAPP2 began enrollment in August 2020 and completed enrollment in May 2022 [9]. The study included a similar sub-study to pilot participants returning home following dosing sessions with MDMA-assisted therapy (and not having a post-visit overnight stay).

The demographics and baseline characteristics of study participants in MAPP1 were not significantly different in terms of race, ethnicity, sex, age, a dissociative subtype of PTSD, disability, and CAPS-5 score across study arms, and across the MAPP1 and MAPP2, see Table 7

Table 7: Demographics and baseline characteristics across MAPP1 and MAPP2 - Phase 3 clinical trials[9,14]

	MAPP2		MAPP1	
Characteristic	**MDMA-Assisted Therapy (*n* =53)**	**Placebo with therapy (*n* =51)**	**MDMA-assisted therapy (*n* =46)**	**Placebo with therapy (*n* =44)**
Age (years), mean (s.d.)	38.2 (11.0)	40.0 (9.6)	43.5 (12.9)	38.2 (10.4)
Sex assigned at birth, *n* (%)				
Male	21 (39.6)	9 (17.6)	19 (41.3)	12 (27.3)
Female	32 (60.4)	42 (82.4)	27 (58.7)	32 (72.7)
Ethnicity, *n* (%)				
Hispanic or Latino	17 (32.1)	11 (21.6)	5 (10.9)	3 (6.8)
Not Hispanic or Latino	36 (67.9)	39 (76.5)	41 (89.1)	40 (90.9)
Race, *n* (%)				
American Indian/Alaska Native	0	2 (3.9)	3 (6.5)	0 (0.0)
Asian	5 (9.4)	6 (11.8)	2 (4.3)	5 (11.4)
Black or African American	5 (9.4)	3 (5.9)	0 (0.0)	2 (4.5)
Native Hawaiian/Pacific Islander	0	1 (2.0)	0 (0.0)	0 (0.0)
White	37 (69.8)	32 (62.7)	39 (84.8)	30 (68.2)
Multiple	6 (11.3)	7 (13.7)	2 (4.3)	6 (13.6)
BMI (kgm–2), mean (s.d.)	26.3 (5.6)	24.7 (4.9)	26.0 (4.8)	24.8 (4.2)
Duration of PTSD (years), mean (s.d.)	16.3 (14.3)	16.1 (12.4)	14.8 (11.6)	13.2 (11.4)
Dissociative subtype of PTSD, *n* (%)	13 (24.5)	11 (21.6)	6 (13.0)	13 (29.5)
Psychiatric disorder, *n* (%)				
Comorbid major depression	49 (92.5)	51 (100)	42 (91.3)	40 (90.9)
Veteran	n/a	n/a	10 (21.7)	6 (13.6)
Suicidal ideation	44 (83.0)	47 (92.2)	n/a	n/a
Trauma history, *n* (%)				
Developmental trauma events	49 (92.5)	43 (84.3)	40 (87.0)	36 (81.8)
Combat exposure	9 (17.0)	6 (11.8)	6 (13.0)	5 (11.4)
Veteran status	9 (17.0)	7 (13.7)		
Multiple trauma events	40 (75.5)	45 (88.2)	41 (89.1)	38 (86.4)
Pre-study PTSD medication, *n* (%)				
Paroxetine	1 (1.9)	1 (2.0)	3 (6.5)	3 (6.8)
Sertraline	15 (28.3)	14 (27.5)	8 (17.4)	9 (20.5)
Pre-study therapy, *n* (%)				
Cognitive behavioral therapy	15 (28.3)	14 (27.5)	12 (26.1)	22 (50.0)
Eye movement desensitization reprocessing	17(32.1)	18(35.3)	17 (37.0)	13 (29.5)
Group therapy	9 (17.0)	15 (29.4)	19 (41.3)	14 (31.8)
Prolonged exposure therapy	2(3.8)	0	1 (2.2)	0 (0)
Psychodynamic therapy	15(28.3)	11(21.6)	11 (23.9)	10 (22.7)
Other	41 (77.4)	42 (82.4)	41 (89.1)	38 (86.4)
None	n/a	n/a	1 (2.2)	1 (2.3)
Cognitive processing therapy	1 (1.9)	1 (2.0)	n/a	n/a
Holotropic breathwork	0	3 (5.9)	n/a	n/a
Dialectical behavioral therapy	4(7.5)	2(3.9)	n/a	n/a
Baseline CAPS-5 total severity score, mean (s.d.)	39.4 (6.6)	38.7 (6.7)	44.0 (6.01)	44.2 (6.15)
Baseline PTSD severity, *n* (%)				
Moderate (CAPS-5 score 28–34)	13 (24.5)	15 (29.4)	n/a	n/a
Severe (CAPS-5 score ≥35)	40 (75.5)	36 (70.6)	n/a	n/a
Baseline SDS modified score, mean (s.d.)	n/a	n/a	6.8 (2.07)	7.4 (1.63)
Baseline C-SSRS score, mean (s.d.)				
Suicidal ideation	0.4(0.8)	0.3(0.6)	n/a	n/a
Ideation intensity	3(5.5)	2.8(5.3)	n/a	n/a
Lifetime C-SSRS, *n* (%)				
Positive lifetime suicidal ideation	n/a	n/a	42 (91.3)	41 (93.2)
Serious lifetime suicidal ideation	n/a	n/a	20 (43.5)	17 (38.6)
Positive lifetime suicidal behavior	n/a	n/a	16 (34.8)	13 (29.5)
Baseline BDI-II total score, mean (s.d.)	25.4(11.9)	25.5(11.3)	30.5 (13.1)	34.9 (12.6)
ACE questionnaire score, mean (s.d.)	4.8(2.9)	4.5(2.7)	5.0 (2.7)	5.0 (2.9)
Prior report of MDMA use, *n* (%)				
Lifetime reported use	22(41.5)	26(51.0)	18 (39.1)	11 (25.0)
Reported use in the past 10 years	13(24.5)	18(35.3)	9 (19.6)	10 (22.7)

For MAPP1, at the primary endpoint, from Baseline to two months after three blinded dosing sessions, there was a significantly more significant mean change in CAPS-5 total severity score in the MDMA-assisted therapy group, with a reduction of 24.4 (SD: 11.6), compared to the therapy paired with an inactive placebo group in which CAPS-5 score was reduced by 13.9 (SD: 11.5), $p<0.0001$, $d=0.91$.

For MAPP1, in the MDMA group, 88% of participants reported a clinically significant reduction in CAPS-5 total severity, and 67% of participants no longer met PTSD diagnostic criteria; compared to the placebo group, 62% reported a clinically significant decrease in CAPS-5 total

severity and 32% no longer meeting PTSD diagnostic criteria. This finding was supported by a positive secondary efficacy in reducing clinician-rated functional impairment. At the Primary Endpoint, there was a significantly greater mean change in SDS scores in the MDMA group, a reduction of 3.1 (SD: 2.6), compared to the placebo group, a reduction of 2.0 (SD: 2.4), p=0.0116, d=0.43. There were no new safety signals found in MAPP1, including no increase in reported adverse events of special interest in the categories of suicidal ideation or behaviour, cardiovascular, or abuse potential in the MDMA group as compared to the therapy with the placebo control group. [57]

For MAPP2, in the MDMA-AT group, 86.5% of the participants were responders with a clinically meaningful improvement at 18 weeks after baseline, defined as a $\geq$ 10-point reduction in CAPS-5 total severity score, versus 69% in the placebo with therapy group[9].

5.4 Pharmacology in Humans

Common AEs of MDMA reported in non-sponsor-supported Phase 1 studies in healthy volunteers include elevation in blood pressure and heart rate, increased anxiety or dysphoria, and dilated pupils. Some reports indicated decreased rather than increased alertness. Other common AEs reported in controlled studies of MDMA include reduced appetite, dizziness, tight jaw, bruxism (tooth-grinding), disturbance in attention, impaired gait or balance, dry mouth, and thirst. Participants in some studies also reported or exhibited changes in cognition, such as increased speed of thought or thought blocking, facilitated imagination or recall. Other less commonly reported events include paraesthesia (unusual body sensations) such as tingling or feeling hot or cold. MDMA can produce anxiety in healthy volunteers. These effects are transient and dissipate as drug effects wane.

5.4.1 Pharmacokinetics and Product Metabolism in Humans

The maximum proposed clinical dosing regimen consists of three divided single-dose exposures to racemic MDMA spaced approximately a month apart with an 80 mg or 120 mg initial dose followed by a 40 mg or 60 mg supplemental dose, administered 1.5 to 2 hours after initial dose at Cmax. Mean (%CV) Cmax and AUC at 125 mg MDMA HCl is 223.5±38.5 ng/mL (N=136) and mean AUC: 948±172.9 ng*h/mL (N=136). The clinical dosing regimen proposed by MAPS does not reach a steady state, as the intended usage is up to three single split exposures with at least two weeks of washout between doses.[15]

Onset of MDMA effects occurs 30 to 60 minutes after oral administration of 75 to 125 mg, peak effects appear 75 to 120 minutes post-drug, and duration of effects lasts from 3 to 6 hours with most effects returning to baseline or near-baseline levels 6 hours after final drug administration. Self-reported duration of effects may increase as the dose of MDMA increases. In a repeated dose study, an initial dose of 50 mg MDMA HCl, followed two hours later by a second dose of 100 mg MDMA HCl, does not significantly extend the duration of measurable physiological or subjective effects in comparison to a single dose of 100 mg MDMA HCl. Orally administered MDMA has a half-life of 7 to 9 hours in humans and the half-life is non-

significantly extended from 10.41 hours to 10.48 hours if an additional dose is administered 2 hours after an initial dose. MDMA and its metabolites have been found in oral fluid samples at much higher concentrations than in plasma, for 24 to 48 hours for the former and 12 to 47 hours for the latter after oral administration of 1 to 1.6 mg/kg MDMA HCl.

The potential for MDMA to be an inhibitor or substrate of human transporters was evaluated in a validated in vitro system. This in vitro interaction study demonstrated that MDMA: is not a substrate for BCRP, MDR1, OATP1B1, and OATP1B3; is not an inhibitor of BCRP, BSEP, MDR1, OATP1B1, and OATP1B3 at up to 500 µM concentrations; and is not an inhibitor of MATE2-K, OAT1, and OAT3 at up to 50 µM concentrations. In vitro MDMA is an inhibitor of MATE1 (IC50 of 6.90 µM), hOCT1 (IC50 of 2.15 µM), and hOCT2 (IC50 of 1.91 µM). To predict whether a drug has the potential to inhibit these transporters, the unbound Cmax/IC50 value must be greater than 0.1. Previous data demonstrates that the unbound fraction of MDMA in human plasma to be 49% at therapeutically active concentration (200 ng/mL) [560] and the Cmax mean to be 223.5±38.5 ng/mL, using 125 mg MDMA HCl. Based on these findings, unbound Cmax was estimated to be 0.48 µM.

MDMA has not been studied in humans with i.v administration. As such, absolute or relative bioavailability is unknown. At a dose of 75mg, MDMA is rapidly absorbed in humans, with an observed Tmax of 1.8 $\pm$ 0.4 hours by the oral route. At higher oral doses of 125mg a slightly longer Tmax 2.4 $\pm$ 1.0 hours was observed.

MDMA has been shown to be partially bound to plasma proteins in humans. The unbound fraction of MDMA in human plasma was measured to be 49% at the therapeutically active concentration (200 ng/mL). The volume of distribution of MDMA at 1 mg/kg was observed to be 5.5±1.1 L/kg (dose range based on weight 43 mg-106 mg) in humans. The volume of distribution of MDMA at 1.6 mg/kg was observed to be 5.5±1.3 L/kg (dose range based on weight 69 mg-150 mg) in humans.

MDMA metabolism in the liver is saturable in a dose-dependent manner and follows non-linear pharmacokinetics. MDMA is metabolized by N-demethylation to the only active metabolite MDA by several enzymes, including CYP2D6 (>30%), CYP1A2, CYP3A4, CYP2C19, and CYP2B6, followed by COMT. The parent compound and MDA are further O-demethylated to HHMA and HHA, respectively. Both HHMA and HHA are subsequently O-methylated mainly to HMMA and HMA. These four metabolites, particularly HMMA and HMA, are excreted in the urine as conjugated glucuronide or sulfate metabolites. It is likely that active doses of MDMA inhibit CYP2D6 function, as measured by examining the effects of MDMA on dextromethorphan metabolism. Inhibition of CYP2D6 by MDMA was demonstrated first in a physiological model derived from data collected after oral administration in humans. O'Mathuna and colleagues present evidence that CYP2D6 activity may not fully recover until 10 days after MDMA.[79,80]

Recently, Vizeli et al. conducted a prospective pooled analysis of eight double-blind, placebo-controlled crossover studies in healthy subjects, including 142 subjects [81,82]. The prespecified primary endpoint of the pooled analysis was to assess the effects of polymorphisms in CYP enzymes on the PK of MDMA in all of the studies. In seven studies, each including 16 subjects, a total of 112 subjects received MDMA at a dose of 125 mg, placebo, one of eight pre-treatments plus MDMA, or the pre-treatment alone. In one study, 30 subjects received MDMA at a dose of 75 mg, placebo, or methylphenidate. Washout periods between treatment periods were at least 7 days. Only data after the administration of MDMA alone without other treatments were included in this analysis, and the washout was considered sufficiently long to exclude any effects of the other treatments on the effects of MDMA alone. All of the studies were registered at ClinicalTrials.gov (NCT00886886, NCT00990067, NCT01136278, NCT01270672, NCT01386177, NCT01616407, NCT01465685, and NCT01771874). The MDMA/MDA AUC6 ratio was greater in subjects with low CYP2C19 or low CYP2B6 function, consistent with a contributing role for both CYP2C19 and CYP2B6 in the N-demethylation of MDMA to MDA in humans and confirming in vitro studies. Additionally, subjects with genetically determined low CYP2C19 function showed a more rapid and greater cardiovascular response to MDMA, although only two subjects with CYP2C19 PM genotype were included in the present study. In contrast to the CYP2C19 genotype, the CYP2B6 genotype altered MDMA concentrations later in time 3–4 h after drug administration. This finding may indicate that CYP2B6 becomes more important when CYP2D6 function decreases over time due to auto-inhibition by MDMA. Overall, polymorphism in CYP1A2, CYP2C19, and CYP2B6 influenced the metabolism of MDMA and genetic polymorphism in CYP2C19 may play a role in clinical toxicity of MDMA. [81,82]

Comparison of pharmacokinetic-pharmacodynamic relationships for MDMA reveals acute pharmacodynamic tolerance. Despite 7 to 9 hours of half-life of MDMA, and persistent high drug levels in the blood, most pharmacodynamic effects of the initial dose rapidly return to baseline within 4 to 6 hours. [15]

Although the hepatic route is thought to be the major route of metabolism in humans with 50% to 75% of the parent compound being metabolized, renal clearance accounts for 8% to 11% of elimination of MDMA and its metabolites. After 1.0 mg/kg MDMA HCl, the majority was excreted in urine as the inactive metabolites HMMA sulfate (13%), followed by DHMA 3-sulfate (9%), and HMMA glucuronide (5%), and only 8% as the parent compound MDMA (55). After 1.6 mg/kg MDMA HCl, the majority was excreted in urine as the inactive metabolites HMMA sulfate (10%), followed by DHMA 3-sulfate (9%), and HMMA glucuronide (4%), and only 11% as the parent compound MDMA. Studies examining metabolism of 100 mg MDMA HCl reported similar excretion values [15]

Metabolites are primarily excreted as glucuronide and sulfate conjugates with some evidence for stereoselective metabolism of the glucuronide and sulfate metabolites. Urinary excretion of the metabolite HHMA after 100 mg MDMA HCl in four men was 91.8±23.8 mol and 17.7% recovery . By contrast, urinary recovery of the major metabolite HMMA after 100 mg was 40%.

Urinary recovery for MDMA and MDA were higher when a second dose of 100 mg MDMA HCl was administered 24 hours after an initial dose of 100 mg MDMA HCl when compared with a single dose. In one study, urinary excretion of the metabolite HMMA exceeded that of MDMA by 33 hours after a dose of 1.6 mg/kg MDMA HCl. Renal clearance (CL) by dose of MDMA HCl administered were 12.8±5.6 L/h at 75 mg, 20.4 12.3 L/h at 100 mg, 13.0±5.4 L/h at 125 mg, and 5.2-11.3 L/h at 150 mg. Oral clearance (CL/F) by dose of MDMA administered was 0.62±0.19 L/h/kg at 1.0 mg/kg (dose range by weight 43 mg-106 mg) and 0.48±0.11 L/h/kg at 1.6 mg/kg (dose range by weight 69 mg-150 mg). To date, there are no known clinical trials quantifying excretion of MDMA into breastmilk in humans.

5.4.1.1 Pharmacokinetic Drug Interactions

Leveraging the data from Phase 1 study (MPKF Study by MAPS) and integrating existing pharmacokinetic data (NIDA data set), Huestis et al., with a MIDD approach, used PopPK and PBPK modelling to describe and predict clinically relevant aspects of MDMA pharmacokinetic.[74] . The PBPK model captured the observed MDMA pharmacokinetics within 1.25- fold for all studies evaluated and within the twofold bound of prediction accuracy and model fidelity. AS MDMA is a strong CYP2D6 MBI (time-dependent), a PBPB modelling approach was applied to stimulate clinical split-dosing regimens, taking CYP2D6 and transporter inhibition into account. Simulated pharmacokinetics for the proposed 120mg and 1280 MDMA HCl clinical doses suggest minor differences when the dose is administered as a split dose over 1.5-2h compared to a single dose, as overall exposure remained similar. The model investigated CYP2D6-mediated DDIs with MDMA as a precipitant. Using atomoxetine as a sensitive CYP2D6 substrate in the model, a sensitivity analysis was completed to predict AUC and Cmax GMR across a range of fm_{CYP2D6} (0.05-0.95). A weak interaction was predicted at fm_{CYP2D6} = 0.05, moderate at fm_{CYP2D6} =0.20 and strong interaction for substrates with fm_{CYP2D6} ≥0.75 [74]. Thereby demonstrated that MDMA is a potent CYP2D6 inhibitor and care should be taken when co-administering with other substrates.

MDMA reversibly inhibits CYPD26 and decreases CYP3A4 activity, with CYP2D6 function normalizing after 10 days. MDMA increases CYP1A2 activity, as measured by caffeine challenge, by 20% to 40% when CYP2D6 is saturated or inhibited. Sarparast et al conducted a systematic review of published randomized controlled studies with healthy adults, epidemiological studies and case reports to understand the interactions between MDMA and medications from several psychiatric drug classes: Adrenergic agents, antipsychotics, anxiolytics, mood stabilizers, NMDA antagonists, psychostimulants, and several classes of antidepressants.[21]

Pindolol, a mixed Beta-adrenergic and 5HT1a receptor antagonist, pre-treatment (1 hour prior to MDMA 1.6 mg/kg p.o) reduced MDMA-induced increases in peak heart rate but had no effect on MDM-induced change in mean arterial pressure, body temperature- or adverse events. Another study by the same group (Hysek et al.) examined the combination of carvedilol (50mg p.o.), an alpha 1 and beta-adrenoreceptor antagonist, with MDMA (125mg p.o.) and found a large reduction in MDMA's cardio stimulant and hyperthermic effects without

significantly affecting MDMA's subjective effects[83,84]. Despite the attenuation of MDMA's physiological effects, there was a significant rise in circulating epinephrine and NE. Clonidine (150 ug po) when administered one hour prior to MDMA (125mg po) modulated the exocytotic release of Norepinephrine and reduced MDMA's effects[21].

Pre-treatment of Dopamine D2 antagonist haloperidol (1.4 mg i.v.) 10 minutes prior to receiving MDMA (1.5mg/kg) resulted in reduced well-being, reduced 'oceanic boundlessness', and a higher rate of state anxiety. Overall, researchers noted an alteration in the physiological profile of MDMA's usually pleasurable state to a dysphoric one. [21]

Schmid et al. studied the combination of bupropion XR (titrated to 300 mg p.o. over 7 days) with MDMA (125 mg p.o. administered concurrently with bupropion XR 300 mg on day 7)[46]. There was a moderate reduction in NE as well as an attenuation in heart rate elevation. No other significant cardiovascular, mydriatic, or hormonal changes occurred compared to MDMA alone. Bupropion did affect self-reported subjective complaints from MDMA, although bupropion significantly prolonged the positive mood effects of MDMA. There was an increase in MDMA levels and reduction in the primary metabolites, DHMA and HMMA, suggesting inhibition of CYP2D6 by bupropion. There was also reduction in MDA levels, suggesting inhibition of CYP2B6 by bupropion. MDMA increased bupropion levels when co-administered due to MDMA's autoinhibition of CYP2D6. [21]

de Sousa Fernandes Perna et al. studied pre-treatment with the NMDA-antagonist memantine (20 mg p.o. 2 h prior) to determine if this would alter MDMA's (75 mg p.o.) effects on memory and mood[85]. The researchers hypothesized that memantine pretreatment may potentially reverse MDMA-induced memory impairment since this had been demonstrated in animal models. The study determined that memantine pre-treatment did not affect MDMA-induced acute memory impairment or impact on mood. [21]

Citalopram, paroxetine, and Fluoxetine attenuated the subjective effects of MDMA by ~ 30– ~ 80%, while physiological effects were attenuated by ~ 6– ~ 14% (except for paroxetine, which was on the order of ~ 40– ~ 60%). Both fluoxetine and paroxetine are strong inhibitors of CYP2D6, and plasma concentrations of MDMA were increased despite the effects of MDMA being attenuated. Exploratory studies demonstrated that paroxetine both blunted MDMA-induced immunosuppression, especially cytokine release and may interact with multiple cytochrome P450 enzymes involved in MDMA metabolism. [21]

5.4.2 Pharmacodynamics

MDMA promotes release and inhibits reuptake of monoamine neurotransmitters and directly binds or indirectly activates downstream receptors, with actions on the serotonin system likely responsible for most of its subjective and physiological effects in humans. MDMA is associated

with changes in several neurohormones, with some of these actions likely responsible for some changes in subjective and physiological effects.

Typically, human trials have used doses between 1 and 2 mg/kg, with therapeutic studies using fixed dosing rather than adjusting dosing on a mg/kg basis, to achieve a more consistent subjective response between individuals. The pharmacokinetics of MDMA in humans have been characterized in blood and urine samples using oral doses of up to 150 mg MDMA HCl. MDMA is a triple monoamine reuptake inhibitor, and similar drugs in this class have been found to exert potent anti-depressant activity with a potentially favorable safety profile. MDMA promotes release, inhibits reuptake, and extends the duration of serotonin, norepinephrine, and dopamine in the synaptic cleft to increase serotonergic, noradrenergic, and dopaminergic neurotransmission. MDMA has self-limiting subjective and physiological effects, as previously described.

In addition to neuroendocrine and norepinephrine-mediated effects, MDMA may target similar binding sites on the SERT, as do already approved PTSD medications Paxil and Zoloft, which are both SSRIs. Like the SSRI Prozac, MDMA also inhibits MAO-A to extend the presence of serotonin in the synaptic cleft. Pre-treatment or co-administration studies of SSRIs with MDMA suggest that this combination is safe within controlled settings and that it attenuates most effects of MDMA. SSRIs appear to attenuate or eliminate most subjective, physiological, and immunological effects of MDMA, likely due to competition for binding sites on the SERT, which may prevent transporter-mediated serotonin release. Additional impacts of each SSRI beyond reuptake inhibition on production, release, and serotonin degradation are likely responsible for variations between SSRI co-administration findings.

The role of serotonin release on the potential therapeutic effects of MDMA-assisted therapy has yet to be investigated; however, reduced feelings of sociability and closeness to others after paroxetine pre-administration suggests that serotonin release is at least partially involved in prosocial effects that are thought to be therapeutically relevant. These subjective effects are predominately mediated by direct or indirect action on 5HT2A receptors with at least one study concluding that the effects of MDMA on positive mood are at least due in part to 5HT2A receptor activation. Findings suggest that the 5HT1A receptor was partially involved in producing the subjective effects of MDMA. A study using receptor-enriched brain mapping of functional connectivity reported that changes in functional connectivity in 5HT2A-receptor-enriched maps were associated with increased reports of having a spiritual experience.

Some MDMA effects on human mood and anxiety may be attributed to dopamine release based on the finding that pre-treatment with haloperidol, a dopamine receptor antagonist with partial selectivity for the D2 receptor subtype, diminished MDMA-induced positive mood, and increased anxiety, though haloperidol alone was associated with dysphoria. Studies comparing MDMA with the dopaminergic and adrenergic drug methylphenidate and bupropion suggest that dopamine release and inhibition of uptake play a minor role, if any, in producing the effects of MDMA. MDMA, but not methylphenidate, increased trust, openness, and closeness

to others, and bupropion prolonged the course of subjective effects without reducing or enhancing them.

MDMA acutely increases cortisol, prolactin, and adrenocorticotropic hormone concentrations in a dose-dependent manner, whereas growth hormone levels are unchanged by up to 125 mg MDMA HCl. A crossover study comparing the effects of MDMA, and methylphenidate found that MDMA increased serum cortisol while methylphenidate did not. Increases in cortisol and prolactin peak at about 2 hours after MDMA administration. A second dose of 100 mg MDMA HCl, given 4 hours after an initial 100 mg, produces a second increase in cortisol during an interval when cortisol levels are declining, and a dose of 100 mg MDMA HCl, given 24 hours after an initial dose, stimulates a greater release of cortisol but not prolactin. Doses of 0.5 and 1.5 mg/kg MDMA HCl elevated cortisol, and under stressful versus less stressful conditions.[57]

MDMA may affect levels of other hormones. In a study of the effects of 0.5 and 1.5 mg/kg MDMA HCl in eight people, there was a trend for increased levels of the hormone dehydroepiandrosterone (DHEA) after 0.5 mg/kg MDMA HCl, and a significant increase after 1.5 mg/kg MDMA HCl, with peak levels appearing 2 to 3 hours post-drug. These findings suggest a relationship between serotonin release and increased serum cortisol. Two studies have found that MDMA increased AVP. Neither study reported analysis or findings concerning any relationship between AVP levels and the subjective, emotional or social effects of MDMA.

A study applying receptor-enriched mapping and functional connectivity in a sample of 20 men reported that 100 mg MDMA HCl activated brain areas assumed to have high 5HT1A density or high levels of SERT density. Despite failing to find a significant change in functional connectivity after MDMA in brain regions high in 5HT2A receptors, Dipasquale and colleagues reported that activity seen in these areas after MDMA were associated with reporting a spiritual experience [86]. This investigation also found a relationship between time course of MDMA effects and changes in functional connectivity in brain areas associated with 5HT1A receptor density, and reported decreased functional connectivity in several cortical areas, including specific areas of the temporal and frontal cortex, and insula, acutely after MDMA. Another investigation of functional connectivity after administering 100 mg to healthy volunteers reported decreased network connectivity in the right insula/salience network [87] with decreased connectivity associated with changes in subjective ratings of trait anxiety and bodily sensations.

5.4.3 Effect on the Central Nervous System

Early investigations of MDMA in healthy volunteers with PET detected decreased left amygdala activity and increased frontal activity[88]. Subsequent studies in healthy volunteers have found reduced activity in the right amygdala and hippocampus, decreased medial prefrontal cortex coupling with the hippocampus, and reduced right insular/salience network connectivity. Decreased activity in the amygdala may be indicative of reduced reactions to potential threats. MDMA (100 mg MDMA HCl) increased subjective ratings of positive mood in response to

positive memories and decreased negative response to negative memories. Attenuated activity in the left anterior temporal area was detected after MDMA during the worst memory recall.

5.4.4 Behavioral Effects

MDMA increases positive mood and anxiety on measures of alteration in consciousness and subjective effects. People receiving active doses of MDMA, defined as 75 to 125 mg MDMA HCl, experience euphoria, positive mood, vigor, and positively experienced derealization, consonant with early retrospective reports, but also report experiencing anxiety, tension, and dysphoria, as well as concern over losing control over the self. There is evidence that increases in positive mood and anxiety increased with dose. Healthy controls reported greater interpersonal closeness to others. It is uncertain whether the increases in positive and negative mood occurred simultaneously or at different times throughout MDMA effects; evidence from two different teams suggests that peaks in negative mood may precede peaks in a positive mood. People have reported feeling anxious or experiencing negative derealization while under the influence of MDMA, including increased anxiety related to loss of control and experiences of racing or blocked thoughts [57].

MDMA may alter detection and receptivity to expressions of emotion. Findings suggest that MDMA might change the way emotional facial expressions might be processed or the response to them. MDMA improved the accuracy of recognizing expressions of positive mood and was less accurate in recognizing expressions of negative mood, and reduced amygdala response to angry faces to suggest possibly an altered response to expressions of anger.

Despite contradictory findings from a naturalistic study, a controlled trial reported that MDMA impaired the detection of expressions of fear. An fMRI study found that 0.75 and 1.5 mg/kg MDMA HCl reduced signaling in the amygdala in response to angry faces when compared with placebo without changing the response to faces showing fear. MDMA reduced the aversiveness of hearing sounds associated with negative emotions. In addition, and contrary to the finding in the early naturalistic study described above, there is some evidence that MDMA might produce selective difficulty in recognizing faces expressing fear. [57]

MDMA in healthy participants makes social activities more attractive, people more generous toward a friend, and more willing to trust a stranger in a "trust game." Rather than increasing trust indiscriminately, trust was given only to trustworthy actors. Studies in healthy controls, comparing doses between 0.75 and 1 mg/kg and 1.5 to 2 mg/kg, suggesting that the higher dose produced greater prosocial effects than the lower dose, while the lower dose may have increased self-reported loneliness and use of empathy-related language. [57]

MDMA does not acutely affect responses on tasks requiring attention and response to visual stimuli or visually presented words but has been shown to interfere with performance on digit-symbol substitution - a measure of attention, psychomotor speed and visual memory. A dose of 75 mg improved visual tracking speed, but impaired estimating the position of a blocked

(occluded) object in a study of acute effects on skills used for driving cars, though without effects on performance monitoring. Subsequently, a series of studies have thoroughly examined the effects of MDMA on road-tracking and car-following performance in both stimulated and on-the-road driving in normal traffic as part of the Driving Under the Influence of Drugs, alcohol, and medicines (DRUID) research consortium funded by a European Union grant. A single dose of 25 mg, 50 mg, 75 mg, or 100 mg MDMA HCl did not produce any dose- or concentration-related effects on driving performance and was found to be generally safe for driving at therapeutic doses in the absence of sleep loss or alcohol intoxication. In one of these studies, driving tests were conducted between 3 and 5 hours after a dose of 75 mg MDMA HCl. Subjects returned the following day for a repetition of the driving tests between 27 and 29 hours post-MDMA. Although changes in cognitive function and psychomotor skills occurred during peak drug effects, these were not detectable on the following day. Acute effects such as excessive caution and impaired positional memory support refraining from driving or using heavy machinery during dosing sessions. MDMA causes slight changes in visual or auditory perception, including changes in the brightness of colours, sounds seeming closer or farther away, simple visual distortions, and altered time perception. Participants also experienced altered time perception and changes in meaning or significance of perceptions after MDMA. There is little indication that MDMA produces any strong alterations to the sense of self or control over the experience. Women reported experiencing all subjective effects of MDMA more intensely compared to men, but especially those related to perceptual changes. These findings should be taken into consideration when designing a clinical trial [57].

Grob and colleagues noted that in a Phase 1 safety study MDMA was found to cause a significant increase in body temperature in some healthy volunteers[89]. Studies conducted by other researchers reported that doses between 1.5 and 2 mg/kg MDMA HCl produced a slight elevation in body temperature that was not clinically significant [82,90]and this elevation was unaffected by ambient temperature [38,91]. Further, 2 mg/kg produced a slight but statistically significant increases in core body temperature with a mean elevation of 0.6°C, at cool (18°C) and warm (30°C) ambient temperatures [91]. A supplemental dose twice as large as the initial dose of MDMA elevated body temperature, but not beyond what was expected after the cumulative dose.

When compared with placebo, findings from 74 people who were given MDMA in oral doses ranging from 70-150 mg MDMA HCl (1.35-1.8 mg/kg) found that men exhibited a greater elevation in body temperature than women when given the dose of MDMA in mg/kg. Subsequent studies have not confirmed this sex difference. A report in a sample of 17 men and women reported higher oral temperatures in women. Before correction for a number of tests, a study on the effects of serotonin-related genotypes on MDMA reported higher body temperature in people with a variant of the TPH-2 gene. Still, these findings were no longer significant after applying corrections. A review of clinical placebo-controlled laboratory studies conducted without sponsor support found that route of measurement influences variability in body temperature findings, with oral and tympanic, but not axillary, temperatures frequently rising above 38°C into moderate hyperthermia ranges at 125 mg MDMA HCl. [57]

MDMA produces sympathomimetic effects, including elevation in blood pressure and heart rate, first recorded by Downing and replicated by other research teams in the U.S. and Europe. MDMA has also decreased respiratory sinus arrhythmia, the natural variation in heart rate throughout each respiratory cycle. Cardiovascular effects of MDMA typically first appear 30 to 45 minutes after administration, peak between 1- and 2 hours post-drug, and wane 3 to 5 hours after drug administration. Men given the same mg/kg dose of MDMA as women exhibited a significantly greater elevation in blood pressure and heart rate in a study summarizing and pooling data from a series of human MDMA studies. These studies did not report any discomfort or increased distress accompanying cardiovascular effects.

Elevation in blood pressure above 140/90 mmHg occurred in approximately 5% of research participants receiving a single dose of at least 100 mg MDMA HCl in Phase 1 research studies. Peiro and colleagues observed elevation in blood pressure above 150/90 mmHg as well as in all 10 participants given 50 mg followed by 100 mg MDMA HCl 2 hours later. When compared with 100 mg MDMA HCl and placebo given 4 hours apart, two doses of 100 mg 4 hours apart significantly elevated SBP, while other physiological effects were not significantly elevated beyond values seen after a single dose. None of these individuals needed clinical intervention and blood pressure returned to normal as drug effects waned. [57]

An examination of liver function as assessed approximately 1 month after MDMA administration in 166 participants, most of them MDMA-naïve, failed to detect any post-drug changes. The first two MAPS-supported Phase 2 studies (MP-1, MP-2) assessed liver function after completion of two or three blinded dosing sessions. Values that differ from established age-appropriate norms were evaluated for clinical significance. Laboratory assessments of liver function were not conducted after dosing sessions in subsequent sponsor-supported studies and no AEs related to liver function have been reported in these studies. Two participants in the MP-2 study reported two clinically significant abnormal laboratory values. One was an elevation in bilirubin in a subject with a family history of elevated bilirubin (probably Gilbert's syndrome), a benign liver condition in which the liver does not properly process bilirubin, with the elevation occurring after open-label treatment with an initial dose of 125 mg to 150 mg MDMA HCl. A family history of mildly elevated bilirubin is considered an indicator of Gilbert's syndrome. Bilirubin levels can be indicative of decreased liver function, but the liver enzymes were normal at that time, supporting the interpretation that the bilirubin levels were slightly elevated compared to baseline due to hereditary factors. The other abnormal laboratory value, an elevation in erythrocyte sedimentation rate (ESR), a marker of inflammation and not a specific liver function marker, occurred in a subject with a medical history of breast cancer. This value was recorded 3 months after the last administration of MDMA as an unrelated AE.
No clinically significant changes in liver function occurred in MP-1. Values for laboratory tests were within the normal range in MP-1. Phase 1 studies conducted outside of sponsor support involving the administration of MDMA to healthy volunteers have not published any results of liver function after MDMA administration. There have been no reported adverse effects on the liver from these studies.

Cardiovascular Effects as correlating with AVP in blood, was detected in women acutely after 125 mg MDMA HCl administration, and this finding was reproduced in another study reporting that 47.5 mg MDMA HCl caused an acute rise in AVP and a small decrease in plasma sodium, at a time of day when it would not be expected to change, in an all-male sample. Norepinephrine release induced by MDMA leads to indirect activation of the AVP system, likely stimulating secretion of copeptin (CTproAVP), a 39-aminoacid glycopeptide that is a C-terminal part of the precursor to pre-proAVP that directly affects AVP. Heart failure is commonly associated with hyponatremia and is also characterized by increased concentrations of basal AVP and CTproAVP in humans. Intra-cardiac pressures, intra-arterial pressures, angiotensin II, pain, and adrenergic (**α**2) central nervous stimuli can also influence AVP secretion. Increased CTproAVP concentration was described in several studies as a strong predictor of mortality in patients with chronic heart failure and acute heart failure.

Research has assessed acute effects of MDMA on perception and cognition acutely after MDMA, commonly at doses between 75 and 125 mg MDMA HCl (or 0.5 to 1.5 mg/kg). In these studies, acute subjective effects peaked 90 to 120 minutes after oral administration and returned to predrug levels 3 to 6 hours later. Sub-acute effects assessed in controlled and naturalistic studies occurred 1 to 3 days after drug administration but were no longer apparent 7 to 14 days later. A study of variations in serotonin-related genes across a pooled sample reported that people with a variant of the 5HT2A gene reported experiencing more "good drug effect," "trust," "high mood," and "dreaminess," and people with a variant in the 5HT1A gene reported a higher "good drug effect," "closeness to others", and lower ratings of a "bad drug effect". People with a variant of the SERT gene reported greater "fear" and 'depression" after MDMA. A second dose of MDMA 2 hours after the first did not increase subjective effects beyond that of an initial dose, which was interpreted by Peiro and colleagues as an indication of tolerance to these effects. When two 100 mg doses were given 4 hours apart, most subjective effects were comparable to those after a single dose, despite double the amount of plasma MDMA. It is notable that the second dose in this study was identical to the first dose, in contrast to sponsor-supported studies, wherein the second dose was half the size of the initial dose.

5.5 Efficacy of MDMA

MAPS PBC, now known as Lykos Therapeutics, has sponsored multiple studies where the effect of MDMA across various populations has been studied. Of notable mention are the pivotal Phase 3 trial-MAPP1 and confirmatory Phase 3 Trial (MAPP2) that evaluated the safety and efficacy of MDMA-assisted therapy for the treatment of moderate to severe PTSD. MAPP1 and MAPP2 studies started with a screening and preparation phase, including two preparatory sessions and a washout period for participants taking any prohibited psychiatric medications. During the treatment phase, three treatment cycles were planned, each lasting three to five weeks and including an eight-hour medication session, and three weekly ninety -minute integration sessions. Participants took 120mg split dose at the medication session in treatment

cycle 1 and 180mg split dose at the medication sessions in treatment cycles 3 and 3. The total duration of the treatment course was approximately 12 -15 weeks, as captured in Figure 6.

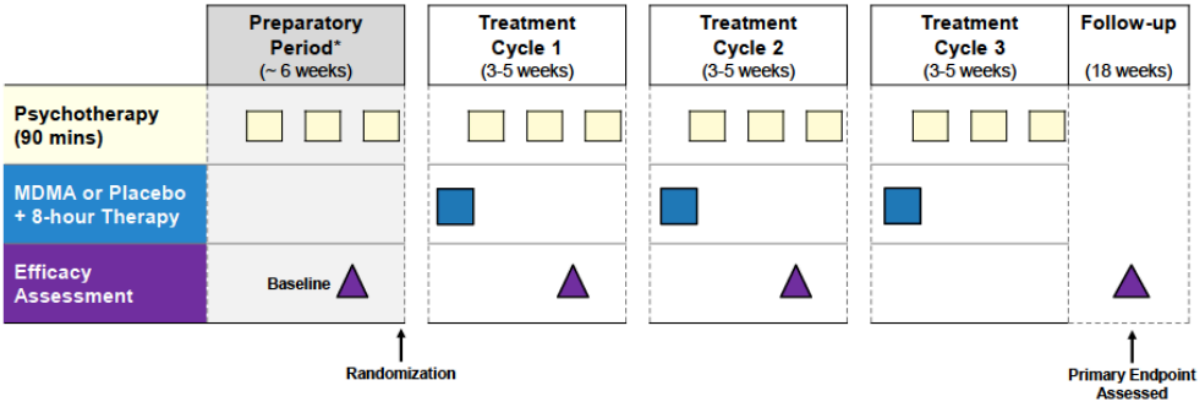

Figure 6 MAPP1 and MAPP2 Clinical Design (Pivotal Phase 3 study design sponsored by MAPS PBC/Lykos Therapeutics)

MAPS PBC/Lykos therapeutics strongly encouraged eligible participants (from MAPP1, MAPP@, MP16, MP17, or MAPPUSX) to enroll in the long-term follow-up study, MPLONG. MPLONG was a multi-center, non-interventional, observational, long-term follow-up study of MDMA-AT. No study drug or therapy was administered. The primary evidence for the durability of the treatment response was from Phase 3 (MAPP1 and MAPP2) participants who enrolled in MPLONG. In total, 60 out of the 90 participants from MAPP1 (MDMA-AT: 30, placebo: 30) and 82 out of the 104 participants in MAPP2 (MDMA-AT: 45, placebo: 37) enrolled in the MPLONG study. Of the 75 participants enrolled in MDMA-AT, 69 completed the study, and 66 of the 67 participants in the placebo group completed all assessments.

5.5.1 MAPP 1 – Pivotal phase 3 clinical trial

During the MAPP1 trial, MDMA significantly attenuated PTSD symptomology, as shown by the change in CAPS-5 total severity score from baseline to 18 weeks after baseline. Mixed model repeated measure (MMRM) analysis of the *de jure estimand* (that is, the effects of the drug if taken as directed) showed a significant difference in treatment arms (n = 89 (MDMA n = 46), P < 0.0001, between-group difference = 11.9, 95% confidence interval (CI) = 6.3–17.4, d.f. = 71) **Error! Reference source not found.**. MMRM sensitivity analysis of the *de facto estimand* (that i s, the effects of the drug if taken as assigned, regardless of adherence) showed a significant difference in treatment arms (n = 90, P < 0.0001, d.f. = 72). The mean change in CAPS-5 scores from baseline to 18 weeks after baseline in the completers (per-protocol set) was −24.4 (s.d. 11.6) (n = 42) in the MDMA-assisted therapy group compared with −13.9 (s.d. 11.5) (n = 37) in the placebo with a therapy group. The effect size of the MDMA-assisted therapy treatment compared with placebo with therapy was d = 0.91 (95% CI = 0.44–1.37, pooled s.d. = 11.55) in the *de jure estimand* and d = 0.97 (95% CI = 0.51–1.42) in the *de facto estimand*. When the within-group treatment effect (which included the effect of the supportive therapy that was administered in both arms) was compared between the MDMA and placebo groups, the effect

size was 2.1 (95% CI = −5.6 to 1.4) in the MDMA group and 1.2 (95% CI = −4.9 to 2.5) in the placebo group.

Over the same period, MDMA significantly reduced clinician-rated functional impairment as assessed with the SDS. MMRM analysis of the *de jure estimand* showed a significant difference in treatment arms (n = 89 (MDMA n = 46), P = 0.0116, d.f. = 71, effect size = 0.43, 95% CI = −0.01 to 0.88, pooled s.d. = 2.53) (Figure 3). The mean change in SDS scores from baseline to 18 weeks after baseline in the completers was −3.1 (s.d. 2.6) (n = 42) in the MDMA-assisted therapy group and −2.0 (s.d. 2.4) (n = 37) in the placebo with therapy group.

MDMA was equally effective in participants with comorbidities that are often associated with treatment resistance. Participants with the dissociative subtype of PTSD who received MDMA-assisted therapy had significant symptom reduction on the CAPS-5 (mean MDMA Δ = −30.8 (s.d. 9.0), mean placebo Δ = −12.8 (s.d. 12.8)), and this was similar to that in their counterparts with non-dissociative PTSD (mean MDMA Δ = −23.6 (s.d. 11.7), mean placebo Δ = −14.3 (s.d. 11.2)). The benefit of MDMA therapy was not modulated by history of alcohol use disorder, history of substance use disorder, overnight stay or severe childhood trauma. Results were consistent across all 15 study sites with no effect by study site (P = 0.1003). In MMRM analysis there was no obvious impact of SSRI history on effectiveness of MDMA.

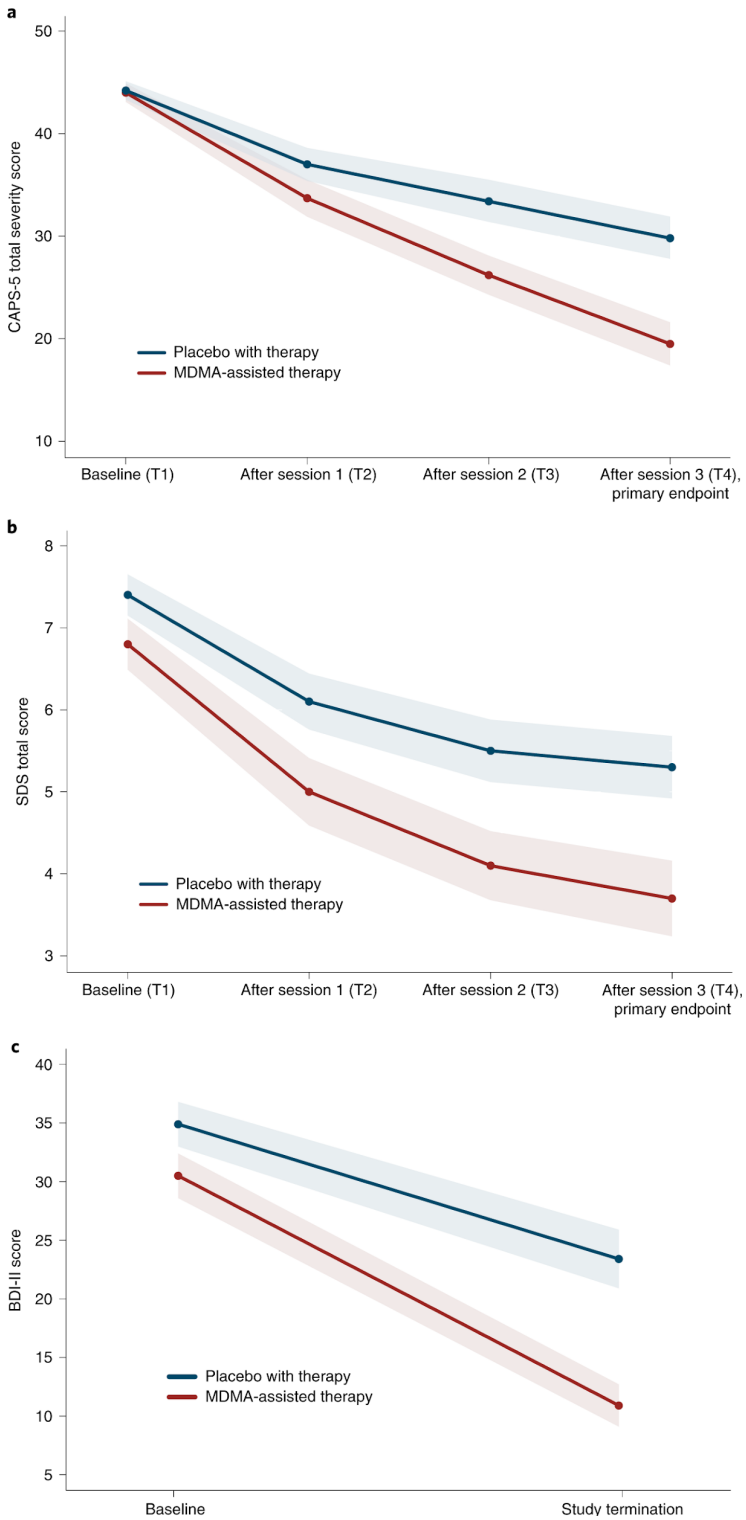

Figure 7: Measures of MDMA efficacy in the MDMA-assisted therapy group and the placebo group.[14]

Fig 3 a, Change in CAPS-5 total severity score from T1 to T4 ($P < 0.0001$, $d = 0.91$, $n = 89$ (MDMA $n = 46$)), as a measure of the primary outcome. Primary analysis was completed using

least square means from an MMRM model. **b,** Change in SDS total score from T1 to T4 (P = 0.0116, d = 0.43, n = 89 (MDMA n = 46)), as a secondary outcome measure. Primary analysis was completed using least square means from an MMRM model. **c,** Change in BDI-II score from T1 to study termination (t = −3.11, P = 0.0026, n = 81 (MDMA n = 42)), as a measure of the exploratory outcome. Data are presented as mean and s.e.m. [14]

MDMA therapy was effective in an exploratory endpoint analysis of the reduction of depression symptoms (using the Beck Depression Inventory-II (BDI-II)) from baseline to study termination of the de jure estimand (mean MDMA Δ = −19.7 (s.d. 14.0), n = 42; mean placebo Δ = −10.8 (s.d. 11.3), n = 39; t = −3.11, P = 0.0026, d.f. = 79, effect size = 0.67, 95% CI = 0.22–1.12).

Clinically significant improvement (a decrease of ≥10 points on the CAPS-5), loss of diagnosis (specific diagnostic measure on the CAPS-5), and remission (loss of diagnosis and a total CAPS-5 score ≤ 11) were each tracked. At the primary study endpoint (18 weeks after baseline), 28 of 42 (67%) of the participants in the MDMA group no longer met the diagnostic criteria for PTSD, compared with 12 of 37 (32%) of those in the placebo group after three sessions. Additionally, 14 of 42 participants in the MDMA group (33%) and 2 of 37 participants in the placebo group (5%) met the criteria for remission after three sessions, see Figure 8.

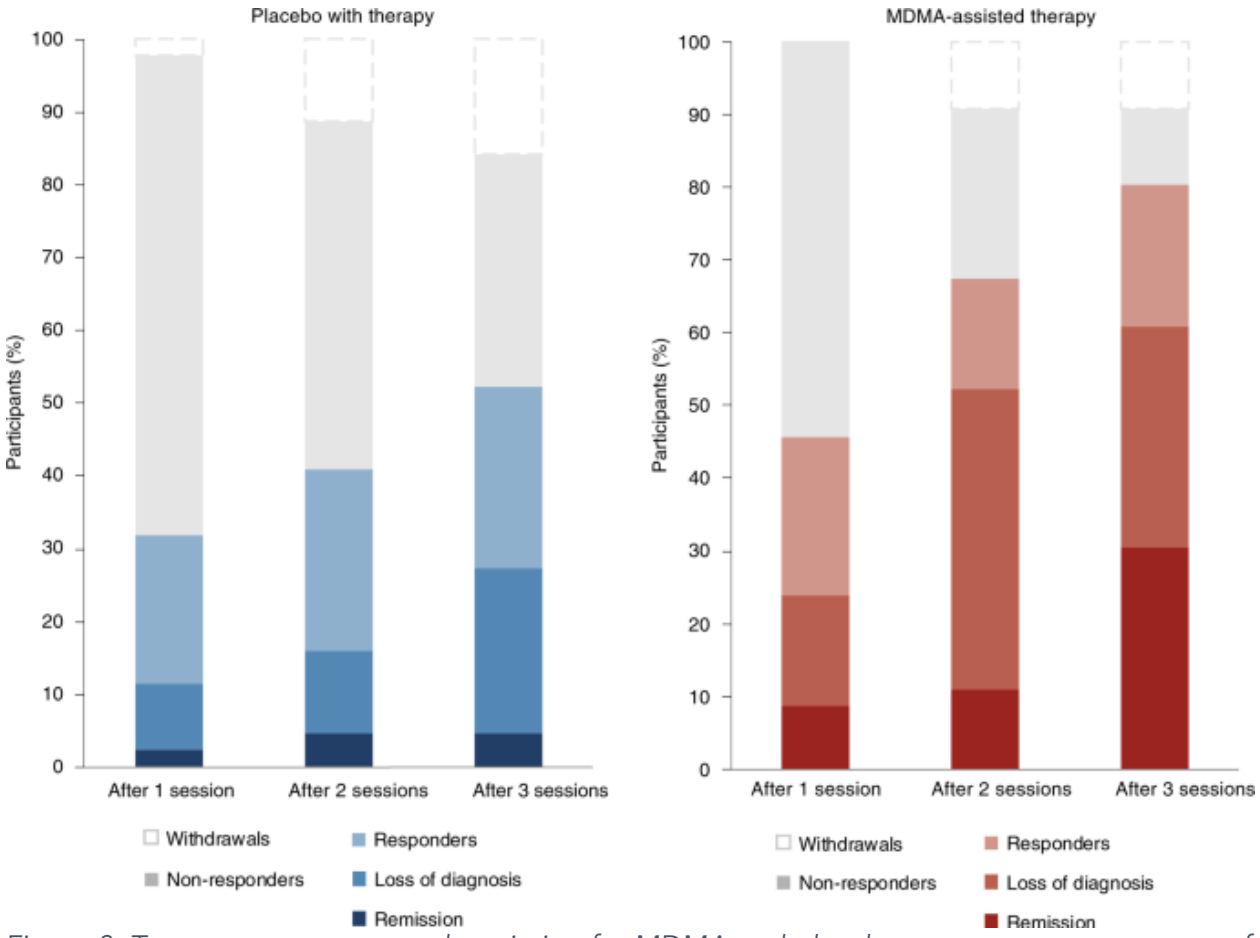

Figure 8: Treatment response and remission for MDMA and placebo groups as a percentage of total participants randomized to each arm (MDMA, n = 46; placebo, n = 44).

Responders (clinically significant improvement, defined as a ≥10-point decrease on CAPS-5), loss of diagnosis (specific diagnostic measure on CAPS-5), and remission (loss of diagnosis and a total CAPS-5 score of ≤11) were tracked in both groups. Non-response is defined as a <10-point decrease on CAPS-5. Withdrawal is defined as a post-randomization early termination.[14]

Data from MAPP 1 was analyzed to provide additional insights into the utility of MDMA-assisted psychotherapy. One such analysis evaluated the different effects of MDMA-AT therapy and therapy with placebo on transdiagnostic outcome measures. It explored the contribution of changes in self-experience to improvement in PTSD Scores[92] Participant symptoms were measured at baseline, at 2 months and after the last experimental session using the 20-item Toronto Alexithymia Scale (TAS-20), the 26-item Self Compassion Scale (SCS), and the 63-item Inventory of Altered Self-Capacities (IASC). The evidence suggests alexithymia and most IASC factors likely mediated the effects of MDMA-assisted therapy treatment on PTSD Symptoms (Table 8). Additional analysis showed baseline alexithymia moderated treatment effects on PTSD symptoms, which warranted examination of CAPS-5 changes scores stratified by baseline TAS-20 subgroup levels (Table 8); and

those with higher alexithymia scores (those worse off) at baseline had greater PTSD symptoms improvement.

Table 8 Change in self-experience scores by treatment group- interaction terms and main effects

	Interaction Term[1]			Main Treatment Effects[2]			Main Treatment Effects adjusted for CAPS-5 change scores[3,5]		
	F-statistic	η^{2} [4]	*p*-value[6]	*F*-statistic	η^{2} [4]	*p*-value[6]	*F*-statistic	η^{2} [4]	*p*-value[6]
TAS-20[5]	$F(2, 76) = 1.17$	0.02	0.32	$F(1, 76) = 15.21$	0.13	0.0002*	$F(1, 74) = 2.48$	0.02	0.12
SCS[5]	$F(2, 76) = 2.85$	0.05	0.06	$F(1, 76) = 22.75$	0.18	< .0001*	$F(1,74) = 7.53$	0.04	0.0076*
IASC[5]									
Interpersonal Conflicts	$F(2, 76) = 0.11$	0.002	0.89	$F(1, 76) = 9.59$	0.06	0.003*	$F(1, 74) = 1.15$	0.0063	0.29
Idealization Disillusionment	$F(2, 76) = 0.77$	0.011	0.47	$F(1, 76) = 6.87$	0.047	0.01*	$F(1, 74) = 1.34$	0.009	0.25
Abandonment Concerns	$F(2, 76) = 0.01$	0.0002	0.99	$F(1, 76) = 4.81$	0.039	0.03*	$F(1, 74) = 0.01$	0.0001	0.92
Identity Impairment	$F(2, 76) = 1.99$	0.0267	0.14	$F(1, 76) = 9.34$	0.0624	0.003*	$F(1, 74) = 0.51$	0.0025	0.48
Self-awareness	$F(2, 76) = 1.00$	0.015	0.37	$F(1,76) = 8.33$	0.063	0.005*	$F(1, 74) = 0.28$	0.0015	0.60
Identity Diffusion	$F(2, 76) = 0.00$	0.0001	1.00	$F(1, 76) = 3.10$	0.0181	0.08	$F(1, 74) = 0.03$	0.0002	0.86
Susceptibility to Influence	$F(2, 76) = 1.04$	0.0099	0.36	$F(1, 76) = 12.12$	0.0579	0.0008*	$F(1, 74) = 1.42$	0.005	0.24
Affect Dysregulation	$F(2, 76) = 0.52$	0.0086	0.60	$F(1, 76) = 5.09$	0.0422	0.03*	$F(1, 74) = 0.06$	0.0004	0.80
Affect Instability	$F(2, 76) = 0.79$	0.0118	0.46	$F(1, 76) = 4.68$	0.035	0.03*	$F(1, 74) = 0.04$	0.0002	0.84
Affect Skill Deficit	$F(2, 76) = 3.81$	0.06	0.03*	–	–	–	–	–	–
Tension Reduction Activities	$F(2, 76) = 0.22$	0.0026	0.80	$F(1, 76) = 5.81$	0.0338	0.02*	$F(1, 74) = 0.28$	0.0014	0.60

[1] Interaction term: treatment x baseline self-experience subgroup levels (e.g., TAS-20, SCS, IASC factor)

[2] Main effects: reported when the interaction term was not statistically significant at alpha level .05; models adjusted for baseline self-experience levels (TAS-20, SCS, or IASC factor) and baseline CAPS-5 dissociative subtype

[3] Model adjusted for baseline self-experience subgroup levels (TAS-20, SCS, or IASC factor), baseline CAPS-5 dissociative subtype, and CAPS-5 change scores

[4] Variance: η^2 = semi-partial eta squared

[5] Abbreviations: CAPS-5 = Clinician-administered PTSD Scale for DSM-5; TAS-20 = Toronto Alexithymia Scale; SCS = Self-compassion Scale; IASC = Inventory of Altered Self-capacities

[6] *statistical significance at $p < .05$

https://doi.org/10.1371/journal.pone.0295926.t001

Table 9 Change in CAPS-5 total severity change scores by treatment group–interaction terms and main effects.

	Interaction Term[1]			Main Treatment Effects[2]		
	F-statistic	η^{2} [3]	*p*-value[5]	*F*-statistic	η^{2} [3]	*p*-value[5]
TAS-20[4]	$F(1, 76) = 4.61$	0.0435	0.04*	–	–	–
SCS[4]	$F(1, 76) = 1.34$	0.0139	0.25	$F(1, 76) = 14.77$	0.1538	0.0003*
IASC[4]						
Interpersonal Conflicts	$F(1, 76) = 0.34$	0.0036	0.56	$F(1, 76) = 17.30$	0.1826	< 0.0001*
Idealization Disillusionment	$F(1, 76) = 0.69$	0.0070	0.41	$F(1, 76) = 15.53$	0.1583	0.0002*
Abandonment Concerns	$F(1, 76) = 0.14$	0.0015	0.71	$F(1, 76) = 17.04$	0.1803	< 0.0001*
Identity Impairment	$F(1, 76) = 0.25$	0.0025	0.62	$F(1, 76) = 16.07$	0.1618	0.0001*
Self-awareness	$F(1, 76) = 0.02$	0.0002	0.88	$F(1, 76) = 17.75$	0.1878	<0.0001*
Identity Diffusion	$F(1, 76) = 0.36$	0.0037	0.55	$F(1, 76) = 16.70$	0.1723	0.0001*
Susceptibility to Influence	$F(1, 76) = 0.98$	0.0102	0.32	$F(1, 76) = 17.89$	0.1861	< 0.0001*
Affect Dysregulation	$F(1, 76) = 0.05$	0.0005	0.83	$F(1, 76) = 18.51$	0.1875	< 0.0001*
Affect Instability	$F(1, 76) = 0.07$	0.0007	0.80	$F(1, 76) = 17.51$	0.1847	< 0.0001*
Affect Skill Deficit	$F(1, 76) = 0.20$	0.0021	0.66	$F(1, 76) = 18.13$	0.1872	< 0.0001*
Tension Reduction Activities	$F(1, 76) = 0.09$	0.0009	0.77	$F(1, 76) = 16.37$	0.1713	0.0001*

[1] Interaction term: treatment x baseline self-experience subgroup levels (e.g., TAS-20, SCS, IASC factor)

[2] Main effects: reported when the interaction term was not statistically significant at alpha level .05; models adjusted for baseline self-experience subgroup levels (TAS-20, SCS, or IASC factor) and baseline CAPS-5 dissociative subtype

[3] Variance: η^2 = semi-partial eta squared

[4] Abbreviations: CAPS-5 = Clinician-administered PTSD Scale for DSM-5; TAS-20 = Toronto Alexithymia Scale; SCS = Self-compassion Scale; IASC = Inventory of Altered Self-capacities

[5] (*) Statistical significance at $p < .05$

https://doi.org/10.1371/journal.pone.0295926.t003

Table 10 CAPS-5 PTSD total severity scores by treatment group and baseline self-experience levels.

	Therapy with placebo			MDMA-assisted therapy					
	Baseline (n = 44)	Visit 20 (n = 40)	Change	Baseline (n = 46)	Visit 20 (n = 42)	Change	N	Between-group difference in change scores	95% CI
Overall Sample	44.23 (6.15)	30.48 (12.56)	-13.32 (1.95)*	43.98 (6.01)	19.55 (13.50)	-24.65 (2.18)*	82	-11.33	-16.59, 6.08
Baseline TAS-20, mean (SD)[1,2,8]									
No alexithymia	45.19 (2.64)	24.61 (2.79)	-20.59 (3.75)*	42.38 (2.60)	17.96 (2.73)	-24.41 (3.64)*	33	-5.24	-15.44, 4.97
Borderline or Alexithymia	44.18 (2.14)	34.98 (2.23)	-9.19 (3.09)	46.23 (2.16)	21.77 (2.25)	-24.46 (3.03)*	49	-16.16	-28.80, -7.52*
Baseline SCS, mean (SD)[1,3,8]									
Moderate or High	42.47 (2.97)	26.01 (3.07)	-16.46 (4.23)*	43.16 (2.56)	20.64 (2.62)	-22.52 (3.61)*	31	-7.48	-18.67, 3.70
Low	45.92 (2.02)	33.48 (2.13)	-12.43 (2.91)*	46.38 (2.21)	20.66 (2.35)	-25.72 (3.08)*	51	-13.85	-22.84, -4.86*
Baseline IASC, mean (SD)[1]									
Interpersonal Conflicts									
≤ median 2.33	45.00 (2.25)	31.73 (2.40)	-13.27 (3.25)*	43.43 (2.32)	18.09 (2.42)	-25.33 (3.29)*	42	-12.80	-22.38, -3.21*
> median	45.03 (2.44)	30.71 (2.51)	-14.32 (3.49)*	47.37 (2.35)	23.93 (2.47)	-23.44 (3.29)*	40	-9.72	-19.79, 0.35
Idealization-Disillusionment[8]									
≤ median 1.84	46.04 (2.52)	30.97 (2.72)	-15.07 (3.64)*	44.03 (2.15)	17.12 (2.25)	-26.91 (3.04)*	40	-12.80	-22.61, -2.98*
> median	44.33 (2.15)	31.51 (2.20)	-12.82 (3.07)*	47.40 (2.49)	26.43 (2.62)	20.96 (3.49)*	42	-8.54	-18.41, 1.34
Abandonment Concerns[8]									
≤ median 2.28	45.00 (2.47)	31.47 (2.66)	-13.54 (3.56)*	42.91 (2.23)	18.17 (2.28)	-24.72 (3.09)*	41	-12.29	-22.12, -2.46*
> median	44.87 (2.21)	30.94 (2.26)	-13.93 (3.15)*	48.04 (2.42)	24.45 (2.61)	-23.59 (3.47)*	41	-10.29	-20.34, -0.23*
Identity Impairment[8]									
≤ median 4.83	45.64 (2.43)	29.94 (2.49)	-15.70 (3.38)*	42.71 (2.17)	15.87 (2.21)	-26.84 (3.01)*	43	-11.87	-21.18, -2.56*
> median	44.49 (2.15)	32.43 (2.30)	-12.07 (3.15)*	48.61 (2.38)	27.88 (2.58)	-20.73 (3.39)*	39	-9.98	-20.41, 0.45
Self-Awareness									
≤ median 3.00	44.27 (2.35)	29.94 (2.39)	-14.33 (3.28)*	42.53 (2.36)	17.53 (2.52)	-24.99 (3.36)*	42	-11.05	-20.62, -1.48*
> median	45.45 (2.40)	32.44 (2.59)	-13.01 (3.53)*	47.61 (2.36)	23.53 (2.43)	-24.09 (3.28)*	40	-11.88	-22.21, -1.54*
Identity Diffusion[8]									
≤ median 1.75	47.00 (2.34)	32.06 (2.44)	-14.94 (3.31)*	43.73 (2.19)	17.19 (2.29)	-26.54 (3.10)*	43	-12.56	-21.92, -3.21*
> median	43.38 (2.29)	30.77 (2.40)	-12.61 (3.31)*	48.09 (2.44)	26.37 (2.55)	-21.71 (3.40)*	39	-9.57	-19.83, 0.69
Susceptibility to Influence[8]									
≤ median 1.78	44.76 (2.33)	31.25 (2.49)	-13.51 (3.34)*	43.76 (2.33)	17.13 (2.50)	-26.63 (3.34)*	40	-13.96	-23.68, -4.24*
> median	45.21 (2.39)	31.17 (2.45)	-14.05 (3.42)*	46.90 (2.37)	24.41 (2.41)	-22.50 (3.26)*	42	-8.78	-18.65, 1.09
Affect Dysregulation[8]									
≤ median 5.80	44.42 (2.34)	27.58 (2.45)	-16.83 (3.34)*	43.30 (2.34)	16.83 (2.44)	-26.47 (3.27)*	41	-10.81	-20.37, -1.24*
> median	45.07 (2.32)	34.38 (2.43)	-10.69 (3.34)	46.52 (2.31)	24.19 (2.42)	-22.33 (3.27)*	41	-12.07	-21.71, -2.44*
Affect Instability[8]									
≤ median 2.50	44.53 (2.41)	29.35 (2.52)	-15.18 (3.42)*	43.82 (2.27)	19.11 (2.35)	-24.71 (3.13)*	44	-10.66	-20.08, -1.25*
> median	45.01 (2.36)	32.67 (2.48)	-12.35 (3.42)*	46.45 (2.52)	22.50 (2.66)	-23.95 (3.60)*	38	-12.03	-22.28, -1.77*
Affect Skill Deficit									
≤ median 3.00	44.16 (2.32)	28.11 (2.37)	-16.06 (3.28)*	42.88 (2.28)	17.39 (2.37)	-25.50 (3.17)*	43	-10.18	-19.67, -0.70*
> median	45.40 (2.31)	34.24 (2.48)	-11.17 (3.37)	47.28 (2.34)	24.10 (2.46)	-23.17 (3.32)*	39	-12.70	-22.60, -2.79*
Tension Reduction Activities[8]									
≤ median 1.78	44.67 (2.48)	28.71 (2.67)	-15.96 (3.60)*	43.73 (2.22)	18.59 (2.30)	-25.14 (3.09)*	41	-10.20	-20.01, -0.39*
> median	44.95 (2.23)	32.79 (2.29)	-12.16 (3.18)*	46.77 (2.49)	23.41 (2.64)	-23.36 (3.54)*	41	-11.82	-21.73, -1.91*

[1] Abbreviations: TAS-20 = Toronto Alexithymia Scale; SCS = Self-Compassion Scale; IASC = Inventory of Altered Self-Capacities; ASC = Altered Self-Capacities

[2] TAS-20 cutoff scores: no alexithymia ≤50; borderline alexithymia (51–60); alexithymia (≥61) (Bagby et al. 1994)

[3] SCS cutoff scores: low (1–2.4); moderate (2.5–3.4); high (3.5–5.0) (Neff 2003)

[4] Change scores are Least Square Means (Standard Errors)

[5] (*) = indicates a *p*-value of < .05 for within-subjects comparison of baseline vs. follow-up scores

[6] (*) indicates a *p*-value of < .05 for between-group subjects' comparison of Therapy with placebo change scores vs. MDMA-assisted therapy change scores

[7] All models adjusted for baseline CAPS-5 Dissociative Subtype (Yes/ No), baseline self-experience score (TAS-20, SCS, or IASC score), change in TAS-20, SCS, or IASC scores, and corrected for multiple comparisons using Tukey's HSD

[8] Baseline levels predicted CAPS-5 change scores

https://doi.org/10.1371/journal.pone.0295926.t004

Results of this exploratory analysis showed both the potential influence of baseline self-experience and MDMA-assisted therapy on self-experience to impact PTSD symptoms, which can be used to guide clinical practice. Further, results found MDMA-assisted therapy improved

self-compassion independent of PTSD treatment which warrants further investigation into potential new applications.

5.5.2 MAPP 2 – Confirmatory phase 3 clinical trial

MDMA-AT significantly attenuated PTSD symptomology versus placebo with therapy, as measured by a reduction in CAPS-5 total severity score from baseline to 18 weeks. Mixed models for repeated measures (MMRM) analysis of the *de jure estimand* showed least squares (LS) mean (95% confidence interval (CI)) change of −23.7 (−26.94, −20.44) for MDMA-AT versus −14.8 (−18.28, −11.28) for placebo with therapy (treatment difference: −8.9 (−13.70, −4.12), $P < 0.001$Figure 9). The Cohen's d effect size of MDMA-AT versus placebo with therapy was d = 0.7; the within-group effect sizes were d = 1.95 for MDMA-AT and d = 1.25 for placebo with therapy. MMRM analysis of the *de facto estimand* revealed an LS mean change (95% CI) in CAPS-5 scores of −23.7 (−26.97, −20.47) for the MDMA-AT group versus −14.8 (−18.24, −11.33) for the placebo with therapy group ($P < 0.001$). MDMA-AT significantly mitigated clinician-rated functional impairment, as measured by a reduction in the Sheehan Disability Scale (SDS) from baseline. MMRM analysis of the *de jure estimand* revealed that the LS mean change (95% CI) in SDS total scores was −3.3 (−4.03, −2.60) with MDMA-AT versus −2.1 (−2.89, −1.33) with placebo with therapy (treatment difference: −1.20 (−2.26, −0.14); P = 0.03, d = 0.4; Figure 9). [9]

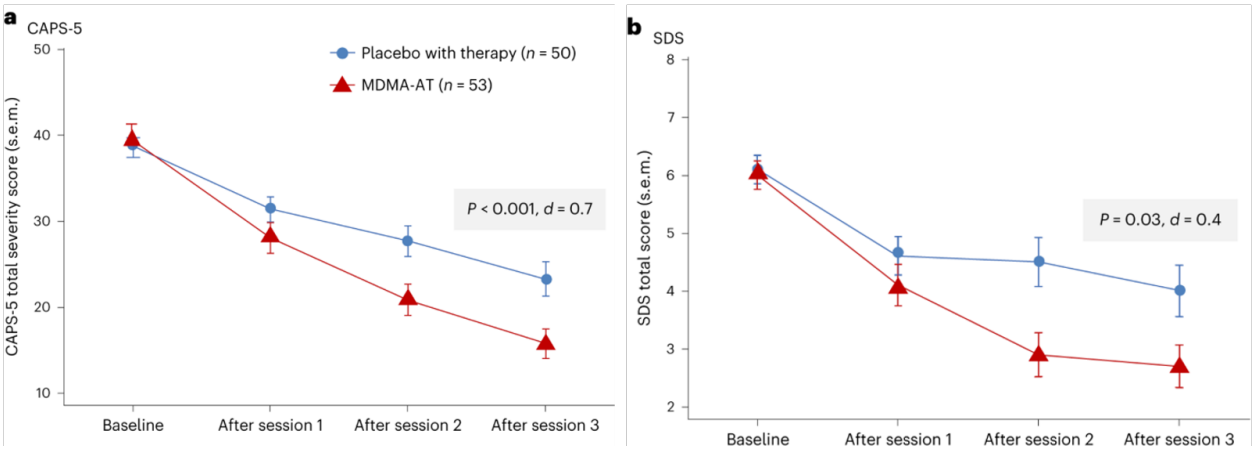

Figure 9: Measures of efficacy in the MDMA-assisted therapy and placebo with therapy groups (MAPP2)

a, LS mean change (±s.e.m.) in CAPS-5 total severity score from baseline to after session 3 (primary outcome) for placebo with therapy (n = 50) versus MDMA-AT (n = 53, $P < 0.001$, Cohen's d = 0.7). b, LS mean change (±s.e.m.) in SDS total score from baseline to after session 3 (key secondary outcome) for placebo with therapy (n = 50) versus MDMA-AT (n = 53, P = 0.03, Cohen's d = 0.4) [9].

Within the MDMA-Assisted Therapy (MDMA-AT) group, 86.5% of participants (45 out of 52) showed a clinically significant improvement 18 weeks after the initial assessment, as indicated by a decrease of 10 or more points in the CAPS-5 total severity score, compared to 69.0% (29 out of 42) in the group receiving placebo with therapy (Figure 10) . By the conclusion of the

study, 71.2% of the MDMA-AT group participants (37 out of 52) no longer fulfilled the DSM-5 criteria for PTSD, in contrast to 47.6% (20 out of 42) in the placebo with therapy group. Additionally, 46.2% of the MDMA-AT group (24 out of 52) and 21.4% of the placebo group (9 out of 42) achieved the criteria for remission (Figure 10). The calculated number of participants needed to treat (NNT) to observe one additional beneficial outcome varied by analysis category: six for both responder and non-responder groups, and four for both loss of PTSD diagnosis and remission groups.[9]

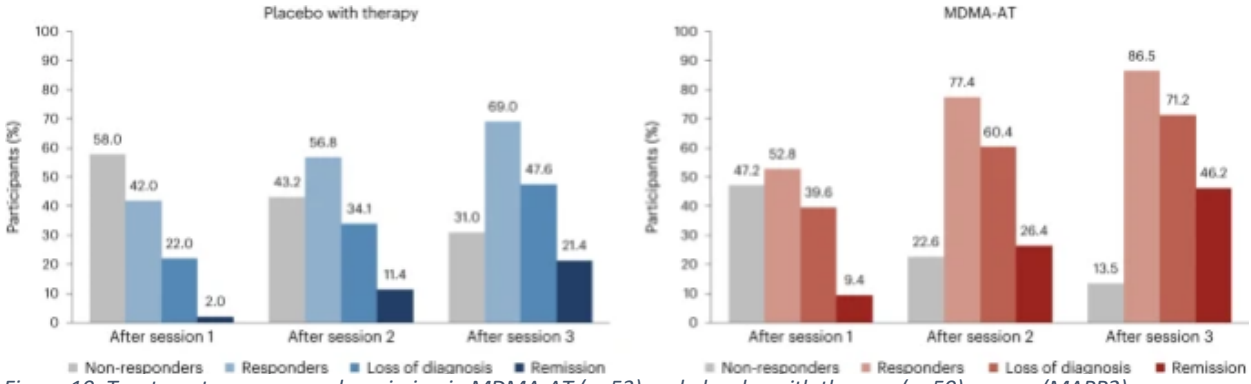

Figure 10: Treatment response and remission in MDMA-AT (n=53) and placebo with therapy (n=50) groups (MAPP2)

A ≥10-point reduction in CAPS-5 total severity score was considered to be clinically meaningful. Responders (≥10-point reduction from baseline), loss of diagnosis (≥10-point reduction from baseline and no longer meeting PTSD diagnostic criteria) and remission (loss of diagnosis and CAPS-5 total severity score of 11 or less) were tracked in both groups as a percentage of participants. Non-responders were defined as any CAPS-5 total severity score change <10-point reduction from baseline. [9]

5.5.3 MPLONG – Long term follow-up study

Figure 11 presents the change from the parent study baseline CAPS-5 total severity score (TSS) for MAPP 2 participants who completed a follow-up PTSD endpoint assessment in MPLONG. The

differences between the treatment groups observed during the parent study (MAPP2) persisted to the long-term follow-up study, which lasted at least 6 months after the last medication session.

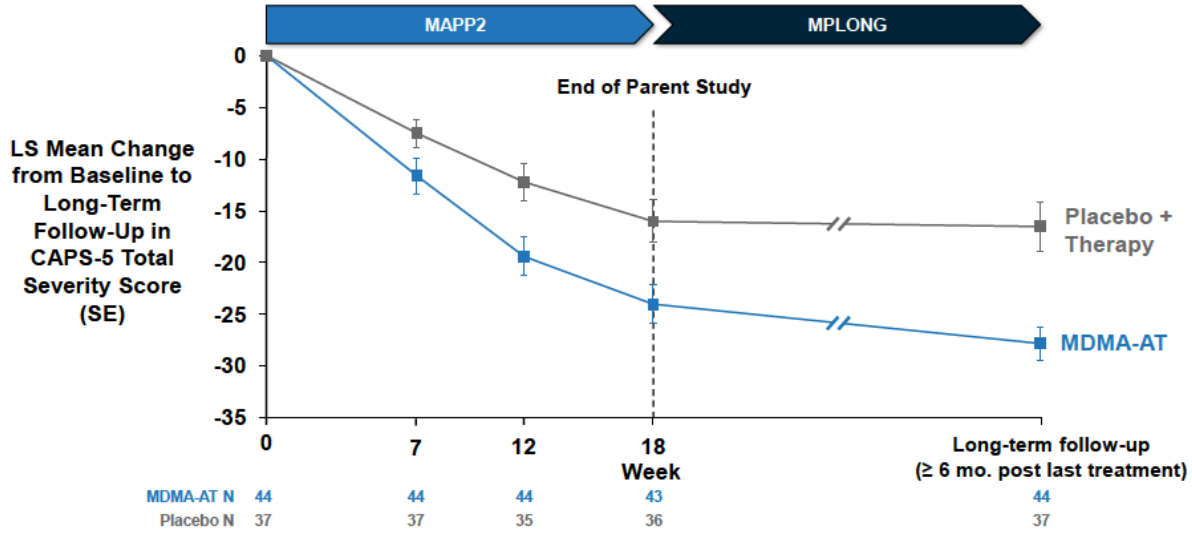

Figure 11 MPLONG Change from parent study baseline in CAPS-5 total severity score (effectiveness subset, MAPP2)

When MPLONG CAPS-5 TSS data for participants were pooled in the MAPP1 and MAPP2 parent studies, a similar separation between the treatment groups was observed (Figure 12). *The effects were sustained* during follow-up visits even after *24 months*.

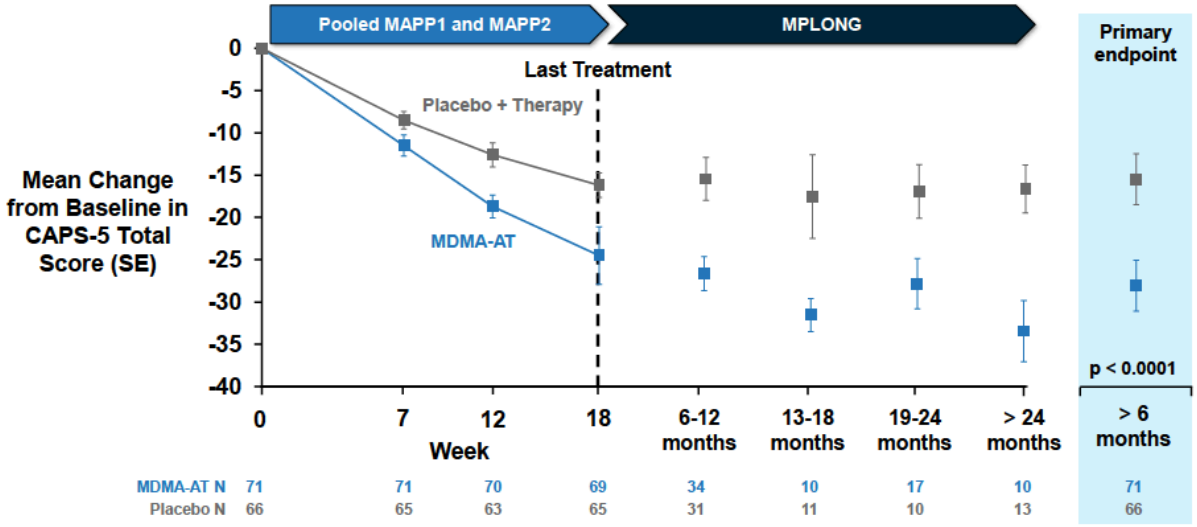

Figure 12 MPLONG change from Phase 3 parent study baseline in CAPS-5 TSS (effectiveness subset MAPP1 and MAPP2)- time since treatment subgroups

5.5.4 PTSD and mild alcohol or cannabis use disorder

A secondary analysis of the Phase 3 (MAPP1) study was conducted, taking into consideration participants who met the DSM-5 criteria for severe PTSD and were permitted to have current mild alcohol or cannabis use disorder. Compared to Placebo and Therapy, MDMA-Assisted therapy was associated with greater decreases in alcohol consumption and risk for hazardous use. [93]The MDMA-AT group, compared to Placebo and Therapy, had a greater statistically

significant reduction (improvement) in AUDIT scores from baseline to study termination [F (80, 1) = 4.20, p = 0.0436; Hedge's g = .45] (Figure 13). No statistically significant difference in AUDIT scores at baseline between treatment groups (p = .10), and AUDIT change scores between treatments were no longer statistically significant after adjusting for baseline AUDIT (p = 0.52). Mean AUDIT scores for the MDMA-AT group were 4.09 (4.16) at baseline and 3.24 (3.36) at study termination (p = .30, Hedge's g = .22), with a change score of − 1.02 (3.52); and for Placebo and Therapy were 2.80 (3.18) at baseline and 3.23 (3.65) at study termination (p = .57 Hedge's g=.13), with a change score of 0.40 (2.70). Mean DUDIT scores for MDMA group were 2.70 (4.31) at baseline and 1.33 (3.14) at study termination (p = .10), Hedge's g = .36); and for Placebo and Therapy were 3.45 (4.46) at baseline and 2.70 (6.33) at study termination (p = .53), Hedge's g = .14). Change in mean DUDIT scores between MDMA-AT vs. Placebo and Therapy were not statistically different at study termination [− 1.36 (3.00) vs. − 0.78, (5.39); F (80, 1) = 0.37; p = 0.5452; Hedge's g = .13, 95% CI = 0.013, 0.89] (Figure 13). There were no statistically significant linear correlations between AUDIT change scores with baseline and change in CAPS-5, BDI-II, and SDS scores in the overall sample.

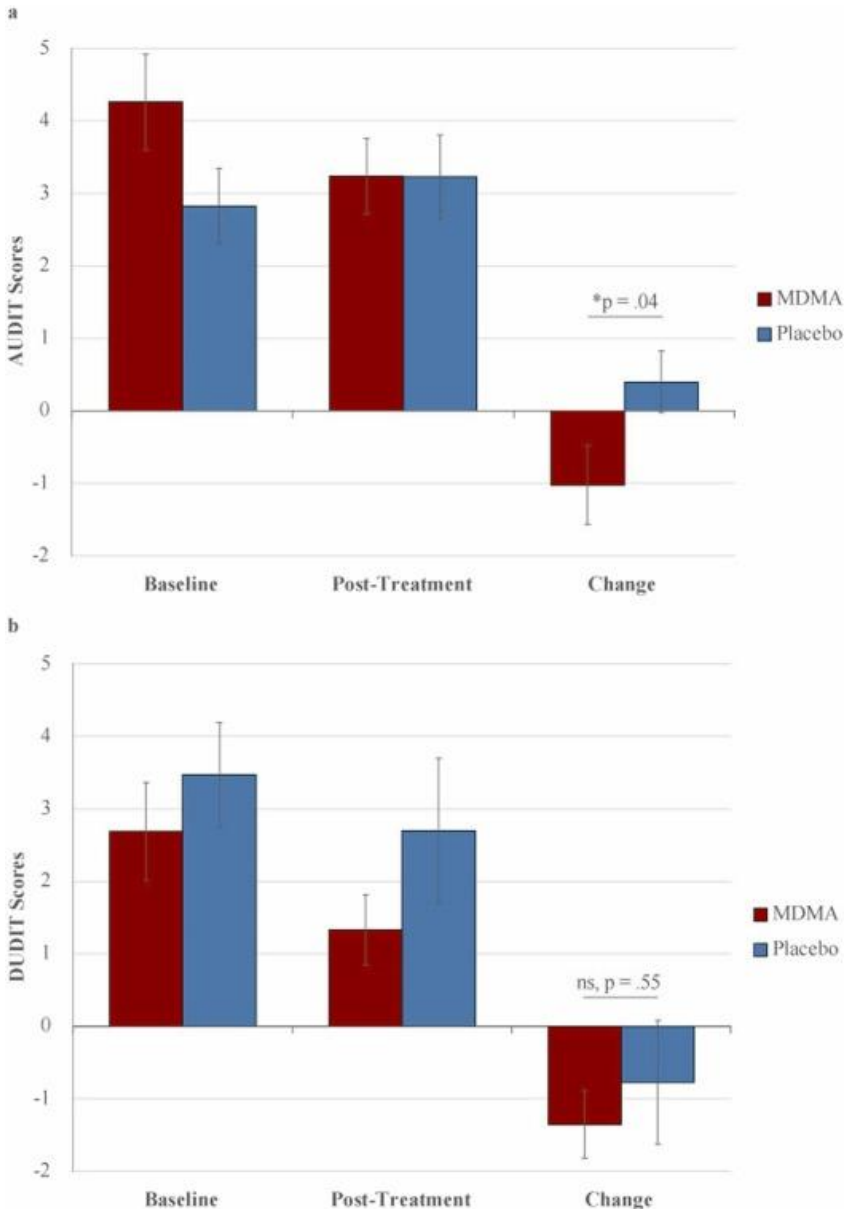

Figure 13: Changes in Alcohol Use Disorder Identification Test (AUDIT)

a. Alcohol use disorder identification test (AUDIT) scores by treatment group, depicted as mean (SEM). Total scores range from 0 to 40. P-value tested treatment group differences for LSMEANS change scores that adjusted for unequal sample sizes, b. Drug Use Disorder Identification Test (DUDIT) Scores by treatment group are depicted as mean (SEM). Total scores range from 0 to 44. P-value tested treatment group differences for LSMEANS change scores that adjusted for unequal sample sizes.[93]

5.5.5 PTSD and eating disorder

Eating disorders (EDs) and posttraumatic stress disorder (PTSD) are highly comorbid, yet there are no proven integrative treatment modalities for ED-PTSD. As part of the secondary analysis of the MAPP1 trial, ninety individuals with severe PTSD received treatment in a double-blind,

placebo-controlled pivotal trial of MDMA-AT. In addition to the primary (Clinician-Administered PTSD Scale) and secondary (Sheehan Disability Scale) outcome measures, the Eating Attitudes Test 26 (EAT-26) was administered for pre-specified exploratory purposes at baseline and at study termination. The study sample consisted of 58 females (placebo = 31, MDMA = 27) and 31 males (placebo = 12, MDMA = 19) (n = 89). Seven participants discontinued prior to study termination. At baseline, 13 (15%) of the 89 individuals with PTSD had total EAT-26 scores in the clinical range (≥20), and 28 (31.5%) had total EAT-26 scores in the high-risk range (≥11) despite the absence of active purging or low weight. In completers (n = 82), there was a significant reduction in total EAT-26 scores in the total group of PTSD participants following MDMA-AT versus placebo (p = .03). There were also substantial reductions in total EAT-26 scores in women with high EAT-26 scores ≥11 and ≥ 20 following MDMA-AT versus placebo (p = .0012 and p = .0478, respectively). ED psychopathology is common in individuals with PTSD, even in the absence of EDs with active purging and low weight. MDMA-AT significantly reduced ED symptoms compared to therapy with a placebo among participants with severe PTSD. MDMA-AT for ED-PTSD appears promising and requires further study.[94]

5.6 Safety of MDMA

During the Phase 3 Clinical trials (MAPP1 and MAPP2) the treatment-emergent adverse events (TEAEs, adverse events that occurred during the treatment period from the first experimental session to the last integration session) that were more prevalent in the MDMA study arm were typically transient, mild to moderate in severity, and included muscle tightness, decreased appetite, nausea, hyperhidrosis and feeling cold[9,14,93]. Importantly, no increase in adverse events related to suicidality was observed in the MDMA group. A transient increase in vital signs (systolic and diastolic blood pressure and heart rate) was observed during MAPP1 in the MDMA group. Two participants in the MDMA group had a transient increase in body temperature to 38.1 °C: one had an increase after the second MDMA session, and one had an increase after the second and third MDMA sessions.

Two participants, both randomized to the placebo group, reported three serious adverse events (SAEs) during the MAPP1 trial. One participant in the placebo group reported two SAEs of suicidal behaviour during the trial, and another participant in the placebo group reported one SAE of suicidal ideation that led to self-hospitalization. Five participants in the placebo group and three participants in the MDMA group reported adverse events of special interest (AESIs) of suicidal ideation, suicidal behaviour, or self-harm in the context of suicidal ideation. One participant in the placebo group reported two cardiovascular AESIs in which underlying cardiac etiology could not be ruled out, see Table 11. One participant randomized to the MDMA group chose to discontinue participation due to being triggered by the CAPS-5 assessments and to an adverse event of depressed mood following an experimental session; this participant met the criterion as a non-responder, which was defined as having a less than 10-point decrease in CAPS-5 score. A lowering of mood did not otherwise follow MDMA sessions.

Table 11: Participants in Phase 3 Clinical Trial (MAPP1) with treatment-emergent SAEs and AESIs

	MDMA ($n = 46$), n (%)	Placebo ($n = 44$), n (%)
SAEs	–	2 (4.5)
Suicide attempts	–	1 (2.3)
Suicidal ideation resulting in self-hospitalization	–	1 (2.3)
AESIs		
Suicidality (total)	3 (6.5)	5 (11.4)
Suicidal ideation	2 (4.3)	3 (6.8)
Intentional self-harm in the context of suicidal ideation	1 (2.2)	–
Suicidal behavior (suicide attempts and preparatory acts) and self-harm	–	1 (2.3)
Suicidal behavior (preparatory acts), self-harm and suicidal ideation	–	1 (2.3)
Cardiac events that could indicate QT prolongation (total)	–	1 (2.3)
Irregular heartbeats and palpitations	–	1 (2.3)
Abuse potential for MDMA (total)	–	–

Suicidality was tracked throughout the MAPP1 study using the Columbia Suicide Severity Rating Scale (C-SSRS) at each study visit. More than 90% of participants reported suicidal ideation in their lifetime, and 17 of 46 participants (37%) in the MDMA group and 14 of 44 participants (32%) in the placebo group reported suicidal ideation at baseline. Although the number of participants who reported suicidal ideation varied throughout the visits, prevalence never exceeded baseline and was not exacerbated in the MDMA group. Serious suicidal ideation (a score of 4 or 5 on the C-SSRS) was minimal during the study and occurred almost entirely in the placebo arm (Figure 14). A more detailed analysis of the MAPS-sponsored studies can be accessed at the MAPS IB[9,57]

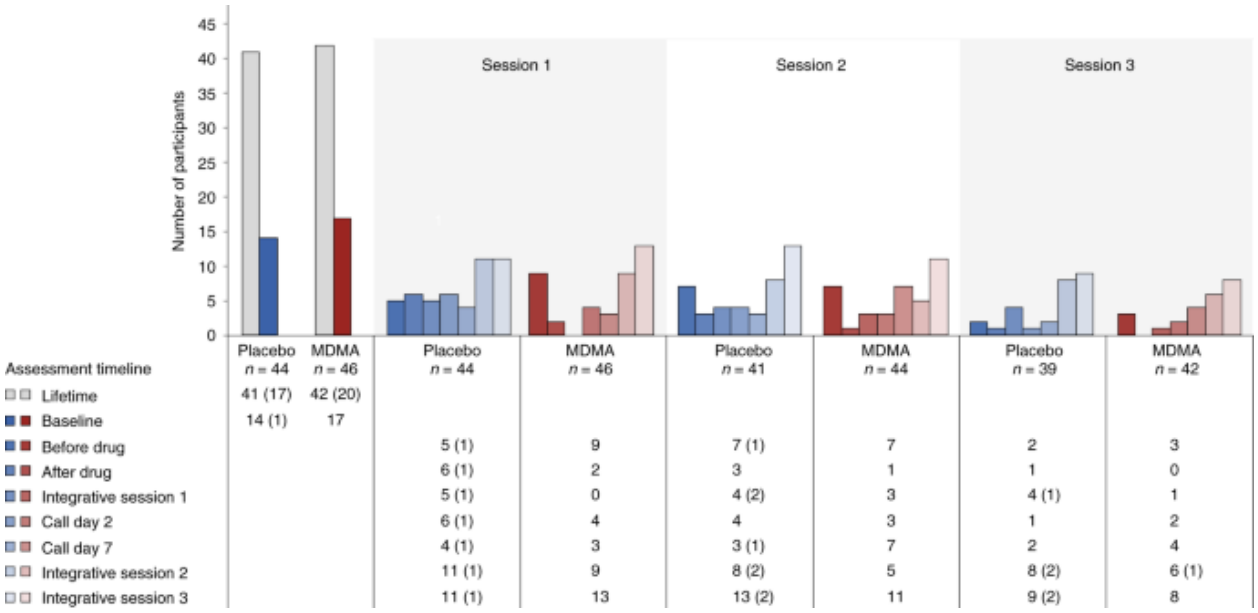

Figure 14: Number of participants reporting the presence of suicidal ideation as measured with C-SSRS at each visit and separated by treatment groups [14]

C-SSRS ideation scores range from 0 (no ideation) to 5. A C-SSRS ideation score of 4 or 5 is termed 'serious ideation'. The number of participants endorsing any positive ideation (>0) is shown by the colored bars and noted in the table below the graph. The number of participants endorsing serious ideation is given in parentheses in the table.[14]

5.7 Vital Signs

Vital sign readings were taken at baseline and post-drug for all MAPS-sponsored studies. In most sponsor-supported studies, tympanic body temperature readings were taken at baseline, then every 60 to 90 minutes after drug administration during each blinded or open-label dosing session, with some differences in collection methods across studies. Peak values during each dosing session were ascertained for all studies, see Table 12. [9,57]

Table 12: Vital Signs of volunteers from MAPS Sponsored Trials[15,57]

MDMA HCl Dose	N	Pre-drug Mean (SD) Min/Max	Peak Mean (SD) Min/Max	Final Mean (SD) Min/Max	N (%) with BT 1 ° C above Baseline
Placebo	10	36.4 (0.51) 35.1/37.2	36.9 (0.36) 36.4/37.6	36.6 (0.36) 35.9/37.5	2 (20.0)
25 mg	8	36.4 (0.39) 35.5/37.1	37.2 (0.78) 36.0/38.5	36.8 (0.65) 35.4/38.0	4 (50.0)
30 mg	7	36.2 (0.47) 35.3/36.9	37.0 (0.42) 36.4/37.9	36.6 (0.47) 35.7/37.3	4 (57.1)
40 mg	6	36.4 (0.50) 35.8/37.2	37.1 (0.33) 36.6/37.6	37.0 (0.38) 36.5/37.6	2 (33.3)
75 mg	7	36.6 (0.46) 35.9/37.8	37.1 (0.52) 36.3/37.8	36.7 (0.42) 36.1/37.3	2 (28.6)
100 mg	9	35.9 (1.00) 33.9/37.9	37.0 (0.64) 35.5/38.7	36.5 (0.74) 34.8/38.1	4 (44.4)
125 mg	58	36.5 (0.47) 35.4/37.6	37.3 (0.49) 36.1/38.6	36.9 (0.54) 34.5/38.2	26 (44.8)
Open-label (100-150 mg)	78	36.4 (0.54) 34.3/37.7	37.3 (0.57) 36.0/38.7	36.8 (0.58) 35.2/38.4	39 (50.0)

In MAPS-sponsored PTSD studies MP-1 to MP-12, body temperature above (1°C above pre-drug reading) was detected in 44% (42 of 95) participants who received any dose of MDMA during blinded sessions and 50% (39 of 78) participants receiving 100 mg to 150 mg MDMA HCl during open-label sessions. Note that body temperature above 1°C above the pre-drug reading was observed in 20% (2 of 10) of participants who received a placebo. End-of-session temperature readings were lower than peak drug readings, though they remained above pre-drug measurements. Body temperature increases that were 1°C or more above initial temperature occurred in all dose groups, suggesting a minimal role for dose.

The maximum body temperature observed for any subject receiving MDMA was 38.7°C, observed after a 100 mg MDMA HCl blinded session, and during open-label sessions. No participants required medical intervention to decrease body temperature and values returned to baseline as drug effects waned. Body temperature measured during dosing sessions in sponsor-supported studies of PTSD was commensurate with values seen in Phase 1 clinical trials described above.

In the MAPS study MAA-1, body temperature 1°C above pre-drug reading was detected in 28.6% (2 of 7) participants who received 100 mg MDMA HCl during a blinded session. Body temperature above the 1°C increase predetermined as cause for increased assessment did not occur during open-label sessions with 75 mg or 125 mg MDMA HCl. The maximum body temperature observed for any subject receiving MDMA in MAA-1 was 37.7°C. No participants

required medical intervention to decrease body temperature and values dropped below peak values or returned to baseline as drug effects waned. Body temperature measurements in this sample were similar to those reported in Phase 1 studies and the sample of people with PTSD.

In the MAPS study-Phase 2 clinical trial of MDMA-assisted therapy for anxiety in relation to a life-threatening illness (MDA-1), body temperature rose 1°C above pre-drug reading in 53.8% (7 of 13) of participants receiving MDMA during blinded dosing sessions and in none of the participants given placebo during blinded sessions. Body temperature rose 1°C above pre-drug reading in 52.9% (9 of 17) participants during open-label sessions. Body temperature recorded at end of session was lower than peak body temperature. The maximum body temperature observed for any subject receiving 125 mg MDMA HCl was 39.9°C. No participants required medical intervention to decrease body temperature and values returned to baseline as drug effects waned.

In MPVA-1, vital signs were assessed on the day of MDMA-assisted sessions prior to drug dosing, just before the optional supplemental dose (midpoint), and at the end of the session. The maximum body temperature observed for any subject receiving 75 mg or 100 mg MDMA HCl was 38.4°C and the mean (SD) temperature prior to the supplemental dose was 37.1°C (SD:0.53) in the CSO participant and 37.2°C (SD:0.48) in the PTSD+ participant.
In the MAPS sponsored Phase 3 PTSD study MAPP1, participants in both groups had minor increases (on average <1°C) in body temperature during dosing sessions. The maximum body temperature observed for any subject receiving 80 mg or 120 mg MDMA HCl) was 38.1°C and the mean temperature prior to the supplemental dose was 36.9°C.

In the MAPS-sponsored Phase 3 PTSD study MAPP1 and MAPP@, participants in both groups had minor increases (on average <1°C) in body temperature during dosing sessions. The maximum body temperature observed for any subject receiving 80 mg or 120 mg MDMA HCl) was 38.1°C and the mean temperature before the supplemental dose was 36.9°C. Based on the published literature, MDMA is expected to produce elevations in body temperature. Humans are not susceptible to changes in ambient temperature when given MDMA, exhibiting slight to moderate increases in body temperature regardless of the environment's temperature.

In all sponsor-supported studies to date, blood pressure readings were taken at baseline, with study-specific differences in data collection times after drug administration. Peak values during each dosing session were ascertained for studies MP-1 through MP-12. In MP16, MP17, and MPVA-1, peak values were collected just prior to the optional supplemental dose (midpoint). The final or endpoint was recorded as the final value at a relatively set time (MP-8, MP-12, MP16, MP17, MPVA-1), as the final value available, or with varying timepoints (MP-1). MP-1 and MP-2 reported two pre-drug values (15 minutes and 5 minutes before dosing) and the results of these measurements were averaged, whereas all other studies reported a single time point pre-drug. Average post-drug values served as the final value for MP-2. If systolic blood pressure (SBP) rose above 160 mmHg or if diastolic blood pressure (DBP) rose above 110 mmHg, additional measurements were collected in studies MP-8, MP-12, MP-9, and MP-4. In

MAA-1, if SBP rose above 180 mmHg or if DBP rose above 110 mmHg, each duration above the pre-determined cut-off was collected. If SBP rose above 180 mmHg and DBP rose above 120 mmHg, each timepoint above the pre-determined cut-off was collected in MDA-1. The cut-off for blood pressure (as SBP/DBP) in MP-2 was >160/110 mmHg. Clinical signs and symptoms were monitored, and more frequent readings were collected in cases where readings were above the cut-off. Candidates with hypertension were excluded from participation in early sponsor-supported studies, but more recent studies have allowed enrollment of participants with well-controlled hypertension. For example, in MP-8, four participants with hypertension controlled by medications were permitted to enroll after completion of carotid ultrasound and nuclear stress test (per protocol) in addition to usual medical screening for the study. MAPS-sponsored investigator brochure provides a more detailed understanding of the changes in vital signs, including blood pressure.

Data from MAPS sponsored clinical trials suggest a dose-dependent transient increase in Systolic blood pressure, heart rate and to a lesser extent diastolic blood pressure in participants that received MDMA, a finding that is supported by data from literature. Both highest peak and maximum duration above clinically important thresholds in cardiovascular parameters were observed in 125 mg MDMA HCl sessions.

In MAPP1 and MAPP2, greater fluctuations in BP were seen during experimental sessions 2 and 3 in the participants treated with MDMA, most likely due to the higher doses of MDMA administered. However, these transient elevations did not require clinical intervention.[9,14]

6.0 Summary of Data

MDMA is a psychoactive compound that affects mood, perception, and increases prosocial feelings. Currently, MDMA is listed as a Schedule I controlled substance in the U.S. and is not permitted for medical use outside of approved research settings. Psychotherapists in the U.S. began to use MDMA as an adjunct to therapy in the mid to late 1970s, and narrative accounts describe therapeutic use with an estimated 500,000 doses of MDMA administered during therapy sessions in North America before its scheduling. PharmAla Biotech has trademarked the name and refers to MDMA as Laneo™ MDMA. It is available to interested researchers, contingent to regional regulatory approvals, to pursue clinical trials and support the further understanding of MDMA and its safety and efficacy in varying patient populations across the globe.

MDMA is responsible for a series of dose-dependent physiological effects due to the enhanced neurochemical release of serotonin, norepinephrine, and dopamine, and for indirect effects on hormone secretion, including oxytocin and AVP, which act on different target organs to modulate physiological functions in the body. MDMA is contraindicated in patients with cardiovascular or cerebrovascular conditions where an acute increase in blood pressure may pose a significant clinical concern, such as aneurysmal vascular disease (including the thoracic

and abdominal aorta, intracranial, and peripheral arterial vessels), arteriovenous malformation, or acute stroke or recent history of intracerebral hemorrhage and during or within 14 days following the administration of monoamine oxidase inhibitors (MAOI). The elevation of blood pressure and increased heart rate produced by MDMA, like that produced by other sympathomimetic drugs, can lead to increased risk in people with pre-existing medical conditions.

In comparison to anxiolytics, antidepressants, and atypical antipsychotics, MDMA does not require steady-state levels in the blood to function as a catalyst to therapy with rapid onset in some participants. A limited number of single divided-dose exposures to MDMA, spaced approximately one month apart in the therapeutically active dose range, are sufficient to obtain therapeutic outcomes. This intermittent dosing mitigates AE frequency and improves the risk/benefit ratio of MDMA, which may provide a significant advantage over medications that require daily dosing. Based on the current state of scientific knowledge and the risk/benefit profile of therapeutic doses of MDMA, MAPS concludes that it appears favourable to continue the clinical development of MDMA as a medicine used as an adjunct to therapy.

6.1 Pharmacology

The pharmacology of MDMA is complex; it activates multiple signalling cascades in the body. The current formulation offered by PharmAla Biotech consists of a fixed dose (40mg) of Laneo™ MDMA HCl in gelatin capsules with inert excipients. There is also a matching placebo available.
The clinical dose tested in Phase 3 trials by MAPS in patients with severe PTSD is a divided dose of 120mg administered in the first dosing session (initial dose of 80mg and a supplemental dose of 40mg) and a divided dose of 180mg (120mg initial + 60mg supplemental) administered in the second and third dosing sessions. The supplemental dose and/or dose escalation can be withheld if the initial dose is not tolerated or the participant declines. In humans, onset of MDMA effects occurs approximately 30 to 60 minutes after initial administration, peak effects occur 75 to 120 minutes after administration and duration of effects lasts 3 to 6 hours. In a repeated dose study, an initial dose of 50 mg MDMA HCl, followed two hours later by a second dose of 100 mg MDMA HCl, does not significantly extend the duration of measurable physiological or subjective effects in comparison to a single dose of 100 mg MDMA HCl.

The pharmacokinetic properties of MDMA in humans have been characterized using initial oral doses of up to 150 mg MDMA HCl in humans. MDMA disposition in the body follows nonlinear pharmacokinetics. MDMA is metabolized in the liver by several enzymes. Active doses of MDMA may autoinhibit CYP2D6 function for an extended period, with function normalizing up to 10 days post-MDMA. The enzymes CYP1A2, COMT, and MAO-A are also involved in the metabolism of MDMA. MDMA is metabolized by N-demethylation to MDA. The parent compound and its active metabolite MDA are further O-demethylated to HHMA and HHA, respectively. Both HHMA and HHA are subsequently O-methylated mainly to HMMA and

HMA. These four metabolites, particularly HMMA and HMA, are known to be excreted in the urine as conjugated glucuronide or sulfate metabolites. The elimination half-life of active MDMA doses is 7 to 9 hours. The end of systemic exposure is within 48 hours post-dose.

MDMA is a triple monoamine reuptake inhibitor, which concomitantly promotes carrier-mediated release, inhibits reuptake, and extends duration of serotonin, norepinephrine, and dopamine in the synaptic cleft to increase serotonergic, noradrenergic, and dopaminergic neurotransmission. Additionally, MDMA appears to alter the conformation of the transporters, enabling monoamines to diffuse out of the neuron rather than being actively transported into the presynaptic neuron. MDMA was found to compete with monoamines for sites on the VMAT2, suggesting MDMA also promotes active release of monoamines from vesicular stores, in addition to inhibiting reuptake. MDMA extends the presence of monoamines in the synaptic cleft by inhibiting MAO-A, an enzyme that breaks down monoamines in the synapse. MDMA has self-limiting subjective and physiological effects. Co-administration with an SSRI or SNRI may eliminate or attenuate the effects of MDMA, and these medications should be tapered in line with the investigator's clinical judgment and an approved study protocol.

Brain imaging studies report that MDMA has been shown to acutely decrease activity in the left amygdala and increase activity in the prefrontal cortex. The chief mechanism behind its therapeutic effects is likely to be serotonergic, along with some norepinephrine and to a minor extent dopamine-mediated effect. Indirect, but potentially significant effects of MDMA include association with release or elevated levels of the hormones cortisol, oxytocin, prolactin, and AVP. MDMA likely stimulates secretion of oxytocin into peripheral blood via indirect activation of 5HT1A, 5HT2C, and 5HT4 receptor subtypes, as well as AVP secretion via activation of 5HT2C, 5HT4, and 5HT7 receptor subtypes. Both oxytocin and AVP are implicated in the widespread regulation of behavioral aspects of mood and act on different target organs to modulate physiological functions in the body. Taken together, MDMA has a diverse array of pharmacodynamic effects in animals and humans.

6.2 Toxicology

The toxicity of MDMA has been investigated in numerous animal and *in vitro* studies published in peer-reviewed journals. Intravenous MDMA doses that cause lethality in 50% of the cases, known as the LD50, are 97 mg/kg in mice, 49 mg/kg in rats, 14 to 18 mg/kg in dogs, and 22 mg/kg in monkeys. LD50 varies between different strains of the same animal species, across the sexes, housing conditions, environmental conditions, social interactions with cohabiting animals, exercise levels, and water supply. Translation of these doses to human-equivalent doses should be undertaken with caution due to known non-linearity in pharmacokinetics that appears in a dose-dependent manner. Exposure-based endpoints are more reliable than simple dose conversions based on body surface area.

MDMA appears to alter regulation of serotonergic signaling in the rat brain without producing damage to serotonin axons, based on transient reductions in forebrain (striatum, hippocampus,

and cortex) serotonin and SERT protein levels, in the absence of indicators of CNS neuronal injury/degeneration. MAPS-sponsored definitive GLP follow-up Extended Single-Dose Neurotoxicology studies in the rat and the dog were conducted to study neurotoxicity with modern experimental methods conclusively. Both studies did not find MDMA-related evidence of CNS neurotoxicity based on expanded neurohistopathology through the MTD. In the rat study, microscopic findings included degeneration of myofibers in skeletal muscle associated with infiltration of mononuclear leukocytes or neutrophils at 25 mg/kg/dose (MTD) one day post dosing. Within 7 days after the last repeated dose, these effects were associated with some level of repair or adaption. On this basis, the risk of CNS neurotoxicity with the intended clinical dosing regimen appears to be minimal, with low risk of transient adverse effects in myofibers of skeletal muscle in the periphery.

MDMA has minimal risk of QTc interval prolongation based on a definitive hERG study which found an IC50 of 206 μM with a 170-fold ratio over the expected unbound MDMA plasma concentration in the clinical exposure scenario. There was no effect of the oral administration of MDMA on qualitative or quantitative ECG parameters in an *in vivo* dog cardiovascular study. MDMA has no significant effects on respiratory rate or blood oxygen saturation by pulse oximetry.

MDMA has been demonstrated to be negative for genotoxicity. Consistent with this, despite very high doses of MDMA being tested in preclinical studies, none have reported carcinogenic effects. Rodent fertility, reproductive, and developmental toxicity studies with MDMA have generally found no abnormalities in gestational duration, neonatal birth weights, or physical appearance when exposure occurs during organogenesis through lactation. Repeated dose toxicity studies of adequate duration, fertility, early embryonic development, and embryofetal development studies of MDMA have been completed. Studies in rats and rabbits have not shown direct or indirect harmful effects with respect to reproductive toxicity. These studies established the NOAEL dose to be the highest dose level evaluated at ≤10 mg/kg/day (supratherapeutic dose) in both sexes of the rat for fertility, reproductive performance, and for maternal and developmental toxicity in the rabbit. The NOAEL dose was the highest dose level evaluated at ≤15 mg/kg/day (supratherapeutic dose) for maternal and developmental toxicity in the rat.

6.3 Safety in Humans

6.3.1 Adverse Events

Overall, adverse effects of MDMA were modest and generally have not been associated with serious discomfort in healthy volunteers or in subjects in MAPS-sponsored Phase 2 or Phase 3 studies with PTSD and other indications. Risks posed by sympathomimetic effects of MDMA treatments are addressed in MAPS' clinical trials by excluding people with pre-existing cardiovascular disease, cerebrovascular disease or uncontrolled hypertension, and by monitoring blood pressure, body temperature, and pulse. Common reactions reported in

clinical trials were transient and diminished as drug effects waned during the MDMA-assisted session and over the next 24 hours. Once the drug is fully cleared from the body within 48 hours post-dose, most reactions diminish within 3-4 days post-dose.

In the Phase 3 study MAPP1, the most common related treatment-emergent adverse events (TEAEs) reported more frequently in the MDMA group were muscle tightness (63.0% MDMA vs 11.4% placebo), decreased appetite (52.2% MDMA vs 11.4% placebo), nausea (30.4% MDMA vs 11.4% placebo), hyperhidrosis (19.6% MDMA vs 2.3% placebo), and feeling cold (19.6% MDMA vs 6.8% placebo). No SAEs, AESIs suggestive of abuse potential, or AESIs involving cardiac function that could be indicative of QT interval prolongation or cardiac arrhythmias were reported in subjects who were randomized to the MDMA group in the Phase 3 study MAPP1 (n=46 of 90 treated). Three participants randomized to MDMA vs. five participants randomized to placebo reported AESIs of self-injurious behavior or suicidality.

Consistent with MAPP1, no new major safety issues were reported in MAPP2. Common TEAEs were similar to previous studies and consistent with expected effects of MDMA. Rates of cardiac TEAEs were low, and increases in BP and pulse were mild, transient and consistent with MDMA's sympathomimetic effects. Consistent with PTSD, suicidal ideation was observed in both groups.

In alignment with MAPP1, there were no reports of problematic MDMA abuse or dependence, including in participants with histories of, or current, alcohol and substance use disorders. However, it is important to note that participants with any substance use disorder other than cannabis or alcohol in the 12 months before enrollment were excluded from MAPP2, as were participants with severe or moderate (in early remission) alcohol or cannabis use disorder. However, exploratory findings from the MAPP1 phase 3 trial indicated that MDMA-AT was associated with a significantly more significant reduction in mean Alcohol Use Disorder Identification Test change scores compared to placebo with therapy, suggesting that the effects of MDMA-AT on hazardous alcohol use secondary to PTSD should be further studied.

Yang et al. [95] analyzed seven clinical trials (NCT00353938, NCT00090064, NCT01211405, NCT01793610, NCT04077437, NCT03537014, and Phase 1 clinical trial([96]) with the intent to investigate the efficacy and safety of MDMA-AT in the treatment of PTSD compared with different control groups. They pooled the data of continuous and binary variables separately and calculated Hedges' g standardized mean difference and RRs with their corresponding 95 %CIs. For the four studies administering placebo to controls, the pooled data of RCTs revealed that the CAPS and SDS scores, which reflect PTSD symptomology, were significantly reduced in the MDMA-AT group compared to the placebo with therapy group. However, the combined results bring up some important questions about the therapeutic modality. MDMA administration can result in a series of AEs, including muscle tightness, nausea, decreased appetite, hyperhidrosis, feeling cold, chills, feeling jittery, restlessness, blurred vision, bruxism, nystagmus, and mydriasis. For the three studies with low-dose administration of MDMA to the controls, the results indicated that the high-dose MDMA groups had significantly more

significant improvements in PTSD symptom severity (reduction from baseline in the CAPS score) than the low-dose MDMA group. However, there were no significant differences in depression symptom severity, sleep quality, or symptoms of dissociation measured by the BDI-II, PSQI, and DES-II, in the high and low-dose MDMA-AP groups. In addition, the proportion of patients with a >30 % decrease in CAPS-IV total score was significantly more prominent in the high-dose MDMA-AP group than in the low-dose MDMA-AP group. Regarding the safety of high-dose and low-dose MDMA, the findings revealed a similar risk of AEs, except for jaw clenching/tight jaw, in the high-dose and low-dose MDMA-AP groups. Therefore, compared with low-dose MDMA, high-dose MDMA may improve the symptoms of PTSD to a certain degree, and the dose of MDMA seemed to have little effect on the occurrence of AEs. [95] Similar results were reported by Colcott et al, Murray et al and Simonsson et al. [97–99]

Cihan Atila et al,[100] recently published their findings that offer new insights into the neuroendocrine basis of MDMA-induced hyponatremia. The authors conducted a secondary analysis of four placebo-controlled crossover randomized clinical trials conducted at University Hospital Basel, Switzerland, that included 96 participants. Plasma oxytocin, copeptin, and sodium levels were measured repeatedly within six hours of MDMA intake. A total of 39 participants (41%) received a 100 mg dose of MDMA, and 57 (59%) received a 125 mg dose. At baseline, the mean (SD) plasma sodium level was 140 (3) mEq/L and decreased in response to MDMA by 3 (3) mEq/L. Hyponatremia occurred in 30 participants (31%) with a mean (SD) sodium level of 133 (2) mEq/L. In 15 participants with restricted fluid intake, no hyponatremia occurred, while in the 81 participants with unrestricted fluid intake, hyponatremia occurred in 30 (37%) ($P = .002$) with a difference in plasma sodium of 4 (95% CI, 2-5) mEq/L ($P < .001$) between both groups, suggesting that fluid restriction may mitigate the risk of hyponatremia. At baseline, the mean (SD) plasma oxytocin level was 87 (45) pg/mL and increased in response to MDMA by 388 (297) pg/mL (ie, a mean [SD] 433% [431%] increase at 180 minutes), while the mean (SD) copeptin level was 4.9 (3.8) pmol/L and slightly decreased, by 0.8 (3.0) pmol/L. Change in plasma sodium level from baseline to 180 minutes demonstrated a negative correlation with the changes in oxytocin ($R = −0.4$; $P < .001$) and MDMA ($R = −0.4$; $P < .001$) levels while showing no correlation with the change in copeptin level [100] . Restricting the fluid intake may mitigate hyponatremia, though more studies are needed to validate the data.

Overall, the risks of SARs have been addressed and constrained by limited exposure and drug administration in controlled settings with adequate screening according to eligibility criteria defined in study protocols. To date, only one SAR (exacerbation of pre-existing ventricular extrasystoles) has been reported within MAPS-sponsored clinical studies. The possibly drug-related expected of exacerbation of pre-existing ventricular extrasystoles occurred during open-label treatment with 125 mg MDMA HCl (supplemental dose was withheld), which resolved with full recovery to baseline after the effects of MDMA ceased. The subject was hospitalized for observation and recovered fully after the event, with no cardiac damage.

6.3.2 Suicidal Ideation and Behavior

In MAPS-sponsored clinical trials C-SSRS scores have escalated during the Preparatory Sessions (before any drug administration), which is thought to be a result of the preparatory discussion of traumatic experiences, and/or of participants tapering off long-prescribed medications, such as SSRIs and benzodiazepines. Withdrawal of these medications is known to induce suicidal ideation or behavior in some people. During both non-drug and MDMA-assisted therapy sessions, participants are asked to think about and discuss their experiences, thoughts, and emotions related to their condition. They may experience intense emotional responses to recalling and speaking about this material. As MDMA is only administered in combination with therapy, the distress associated with therapy is unavoidable and is considered by MAPS as a necessary part of the therapeutic process that requires proper facilitation and support from therapists.

Overall, the incidence of serious suicidal ideation or behavior in MAPS sponsor-supported studies was low, occurring in only a few participants post-MDMA treatment and an equivalent number of participants either before dosing or after therapy with placebo, and returning to lower scores while participants were closely monitored. As of October 01, 2023, six participants reported SAEs of suicidality (suicidal ideation and/or behavior) after receiving MDMA in MAPS-supported Phase 2 and Phase 3 studies. All were deemed unrelated to MDMA in the opinion of the investigator and sponsor. Of interest, three participants reported SAEs of suicidality either prior to receiving study drug (n=1, Study MP-2), or after receiving placebo (n=2, Study MAPP1). In aggregate, the comparable incidence across groups demonstrates that these SAEs remain unexpected for MDMA, and the underlying disease presents an alternative explanation for these SAEs.

In the placebo-controlled Phase 3 trial MAPP1, suicidality, as measured by C-SSRS and reported as AE, occurred with equal prevalence in both study arms; the prevalence was not more remarkable in the MDMA arm of the study. There were no reports of positive suicidal ideation or behavior after the first dosing session in participants with a life-threatening illness and only few incidences, nonserious, of positive ideation in adults on the autism spectrum during the study. In MAPP2, MDMA did not appear to increase this risk, and no suicidal behaviour was observed. C-SSRS scores varied throughout the study but never exceeded baseline values for either group. Notably, there were five total events of treatment-emergent C-SSRS scores of 4 or 5: three in the MDMA-AT group and two in the placebo with therapy group. MAPP2 enrolled participants with a history of suicidality but excluded those with a current, serious imminent suicide risk; thus, special attention to this vulnerable population is warranted in future studies.[9]

In the pooled analysis, it was noted that at screening, in the MDMA-AT and placebo groups, 86.9% and 88.4% of participants had any lifetime history of suicidal ideation (defined as a C-SSRS suicidal ideation score of > 0), and 35.4% and 36.8% participants had lifetime suicidal ideation scores of 4 or 5 (considered serious ideation), respectively. In the MDMA-AT and

placebo groups, 27.3% and 30.5% had prior history of suicidal behavior, respectively. The percentages of participants who reported treatment-emergent suicidal ideation were similar in the 2 treatment groups (MDMA-AT: 39.4%; placebo: 44.2%), as were the frequencies of intentional self-injury (MDMA-AT: 3.0%; placebo: 5.3%) (Table 13).[72]

Table 13 TEAEs of suicidal ideation, intentional self-injury, suicidal behavior, suicide attempt, and self-injurious beahvior (MAPP Phase 3 pool)

Preferred Term (PT)	MDMA-AT N= 99 n (%)	Placebo + Therapy (N=95) N(%)
Suicidal ideation	39 (39.4)	42 (44)
Intentional self-injury	3 (3.0)	5 (5.3)
Suicidal behavior	0 (0.0)	2 (2.1)
Suicide attempt	0 (0.0)	1 (1.1)
Self-injurious ideation	0 (0.0)	1 (1.1)

Therapy teams minimize risks by carefully evaluating all participants to determine if there is a current risk of suicidal behavior. Participants with a history of suicide attempts are not excluded unless significant risk of suicidal behavior is present at the time of Screening. When positive serious ideation or behavior occurred after study enrollment, the investigators made follow-up observations of C-SSRS to ensure subject safety and, tracked scores until they returned to non-serious levels. This risk is critical to monitor due to the high prevalence of background events in the psychiatric population.

6.3.3 Immunological effects

Humans exhibit transient immunological changes after a dose of 100 mg, including reduced numbers of CD4 cells, increased numbers of NK cells, and increased levels of immunosuppressive and anti-inflammatory cytokines compared with levels of pro-inflammatory and immunostimulant cytokines. In several respects, these effects are like those that occur with other psychoactive substances, so are not unique to MDMA. Immunological effects last for approximately 24 hours after administration, and most arise indirectly from serotonin release. The significance of these immunological effects remains unclear. Based on results from trials conducted by the sponsor to date, the impact of these effects is expected to be modest. Further evaluation in the placebo-controlled Phase 3 study MAPP1 did not demonstrate an increase in upper respiratory tract infections in the MDMA group compared to the placebo group.

6.3.4 Hepatic effects

Phase 1 studies conducted outside of sponsor support involving administration of MDMA to healthy volunteers have not reported any results of liver function after MDMA administration,

and a recent published examination of safety data in a pooled sample of healthy participants found no changes in hepatic function assessed via standard liver panel. There have been no reported adverse effects on the liver from these studies. The first two sponsor-supported Phase 2 studies (MP-1, MP-2) assessed clinical lab values for safety after completion of two or three dosing sessions. On average, no clinically or statistically significant changes in ALT, as a measure of liver function, occurred in MP-1. All post-treatment values for laboratory tests were within the normal range in MP-1. No MDMA-related AEs related to liver function have been reported in subsequent sponsor-supported studies. Only one participant in the MP-2 study reported a clinically significant hepatic abnormalities, likely due to hereditary factors. The other observed laboratory value (increased ESR) indicated inflammation in a subject with a medical history of breast cancer 3 months after the last administration of MDMA as an unrelated AE. Hepatic function one-month post-MDMA was assessed in nine independent published safety pharmacology clinical trials that were published in a pooled analysis (N=164). These studies found one significant change found in **γ**-glutamyl transpeptidase ($p<0.01$). There were no significant changes in creatinine, GFR, ALT or AST. These results support that on average, MDMA does not influence hepatobiliary function in most people, however there is evidence of a rare idiosyncratic hepatotoxicity.

6.4 Risk Assessment

MDMA is known to transiently increase heart rate and blood pressure in a dose-dependent manner that is generally not problematic for physically healthy individuals. An examination of safety data drawn from MAPS-sponsored Phase 2 and Phase 3 studies of MDMA-assisted therapy detected a dose-dependent increase in SBP and, to a lesser extent, DBP. MAPS addressed the risks of elevated blood pressure by excluding people with pre-existing uncontrolled hypertension and monitoring blood pressure and pulse.

A medium-level risk of exacerbation of psychological distress exists in participants with a psychiatric diagnosis. Data in PTSD patients from the sponsor's Phase 3 clinical trial (MAPP1) indicate TEAEs related to psychological distress were similarly reported across both groups of blinded participants: anxiety (32.6% MDMA, vs 40.9% placebo), depressed mood (10.9% MDMA vs 9.1% placebo), and suicidal ideation (45.7% MDMA vs 47.7% placebo). These commonly reported AEs were observed in both treatment arms, transient in duration, mild to moderate in severity, and overlap with background events associated with PTSD, which might have influenced the frequency of events observed during clinical trials of MDMA-assisted therapy. While these data do not support that psychological distress is a specific risk of MDMA, psychological distress may be exacerbated in participants with moderate to severe PTSD following the onset of MDMA effects until the last effects have dissipated.

In data gathered from sponsor-supported Phase 2 and Phase 3 studies, it was found that compared to placebo, a higher percentage of participants receiving MDMA had peak body temperatures greater than 1°C above baseline. However, there was no strong relationship

between dose of MDMA and peak body temperature or between MDMA dose and elevation above threshold of 1°C above baseline.

The standard genotoxicity battery for MDMA has demonstrated that MDMA is negative for *in vitro* and *in vivo* genotoxicity, both with and without metabolic activation.

There are no data from the use of MDMA in pregnant people.

Common expected AEs were typically observed during dosing sessions but were transient and typically diminished as MDMA was metabolized and excreted over the next 48 hours after dosing. In the Phase 3 study MAPP1, the most common adverse events reported more frequently in the MDMA group were (>20%) muscle tightness, decreased appetite, nausea, hyperhidrosis, feeling cold, (>10%) restlessness, mydriasis, dizziness (postural), bruxism, nystagmus, increased blood pressure, feeling jittery, chest pain (non-cardiac), dry mouth, vision blurred, pollakiuria, intrusive thoughts, vomiting, stress, and musculoskeletal chest pain. AEs were typically self-limiting in MAPS sponsored studies.

It does not appear that MDMA-assisted therapy negatively impacts cognitive function based on data from Phase 2 studies sponsored by MAPS. The sponsor has carefully considered the risks of neurotoxicity and concludes that they are minimal in the intended clinical dosing regimen. This conclusion is supported by empirical and toxicokinetic evidence and is consistent with the lack of CNS toxicity reported in nonclinical MDMA studies.

7.0 Conclusion

MDMA is a molecule with complex pharmacology. Lykos Therapeutics, formerly known as MAPS PBC[101,102], has led efforts to establish the safety and efficacy of MDMA-assisted therapy through multiple Phase 2 and Phase 3 clinical trials in patients with severe PTSD. After receiving breakthrough therapy and fast-track designation for their new drug application (NDA) by the US Food and Drug Administration (FDA), Lykos hoped to receive approval in 2024. However, the Psychopharmacologic Drugs Scientific Advisory Committee meeting held in June 2024 was not convinced, based on the data presented, that the benefits outweighed the risks. Following this, the US FDA, in their complete response letter to Lykos Therapeutics, has asked for additional safety data and a new Phase 3 trial. The effect of MDMA-assisted therapy in other indications, such as Eating disorders, Anxiety in severe illness, social anxiety in autistic adults, alcohol use disorder, schizophrenia, etc, is currently underway.

Based on the current state of scientific knowledge, the risk for subjects meeting criteria for clinical studies who are exposed to MDMA at the single divided-dosing schedule, administered up to 3 times per treatment course, and used in sponsor-supported studies appears to be moderate to low. Across Phase 2 and Phase 3 studies, the overall rates of AEs and reactions are acceptable and generally self-limiting. Safety data from the placebo-controlled Phase 3

trials indicates that common AEs reported at equivalent rates across groups are likely to represent the underlying illness being treated or the expected result of psychological therapy addressing traumatic experiences.

The pivotal Phase 3 trial (MAPP1) and confirmatory phase 3 trial (MAPP2) demonstrated that PTSD symptoms were significantly attenuated by MDMA-assisted therapy and confirmed findings seen in Phase 2 studies. MDMA as an adjunct to supportive therapy was statistically superior for PTSD treatment in CAPS-5 severity scores from Baseline to 2 months after three blinded dosing sessions in comparison to therapy paired with an inactive placebo. Future studies conducted by Lykos Therapeutics are intended to develop further the safety profile of MDMA in the PTSD subject population and subjects with other indications.

Based on the body of evidence in the public domain, MDMA-assisted therapy appears to be a promising treatment method for chronic PTSD. It is hoped that MDMA, with its unique pharmacological mechanisms combined with a novel mode of administration as an integrative, multimodal therapy, can improve upon available PTSD and anxiety treatments in terms of safety profiles, efficacy, and persistence of effectiveness. Based on the previously reported data and the current state of scientific knowledge, MDMA-assisted therapy demonstrates potentially significant benefits that may outweigh the risks of using a 3-session treatment model combined with non-drug therapy sessions for preparation and integration in the treatment of PTSD.

8.0 Bibliography

[1] Health TLG. Mental health matters. *Lancet Glob Heal*. 2020;8(11):e1352. doi:10.1016/s2214-109x(20)30432-0

[2] Mental Illness - National Institute of Mental Health (NIMH). https://www.nimh.nih.gov/health/statistics/mental-illness. Accessed February 26, 2024

[3] Katz C, Stein M, Richardson JD, Sareen SS and J, Sareen J. A Review of Interventions for Treatment-Resistant Posttraumatic Stress Disorder | IntechOpen. In: Selek S, ed. *Different Views of Anxiety Disorders*. ; 2011:382. doi:10.5772/23234

[4] Sareen J. Post-traumatic stress disorder in adults: Epidemiology, pathophysiology, clinical features, assessment, and diagnosis - UpToDate. UpToDate. https://www.uptodate.com/contents/posttraumatic-stress-disorder-in-adults-epidemiology-pathophysiology-clinical-features-assessment-and-diagnosis. Accessed February 26, 2024

[5] W A. Pharmacotherapy for Post-traumatic Stress Disorder In Combat Veterans. *P & T : a peer-reviewed journal for formulary management*. 2012;37(1):32-38. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3278188/pdf/ptj3701032.pdf

[6] Feduccia AA, Jerome L, Yazar-Klosinski B, Emerson A, Mithoefer MC, Doblin R. Breakthrough for Trauma Treatment: Safety and Efficacy of MDMA-Assisted Psychotherapy Compared to Paroxetine and Sertraline. *FOCUS*. 2023;21(3):306-314. doi:10.1176/appi.focus.23021013

[7] Lee DJ, Schnitzlein CW, Wolf JP, Vythilingam M, Rasmusson AM, Hoge CW. PSYCHOTHERAPY VERSUS PHARMACOTHERAPY FOR POSTTRAUMATIC STRESS DISORDER: SYSTEMIC REVIEW AND META-ANALYSES TO DETERMINE FIRST-LINE TREATMENTS. *Dépress Anxiety*. 2016;33(9):792-806. doi:10.1002/da.22511

[8] Feduccia AA, Jerome L, Mithoefer MC, Holland J. Discontinuation of medications classified as reuptake inhibitors affects treatment response of MDMA-assisted psychotherapy. *Psychopharmacology*. 2021;238(2):581-588. doi:10.1007/s00213-020-05710-w

[9] Mitchell JM, G MO, Kolk B van der, Shannon S, Bogenschutz M, Gelfand Y, et al. MDMA-assisted therapy for moderate to severe PTSD: a randomized, placebo-controlled phase 3 trial. *Nat Med*. Published online 2023:1-8. doi:10.1038/s41591-023-02565-4

[10] Nichols DE, Hoffman AJ, Oberlender RA, Jacob P, Shulgin AT. Derivatives of 1-(1,3-benzodioxol-5-yl)-2-butanamine: representatives of a novel therapeutic class. *J Med Chem*. 1986;29(10):2009-2015. doi:10.1021/jm00160a035

[11] Nichols DE. Entactogens: How the Name for a Novel Class of Psychoactive Agents Originated. *Front Psychiatry*. 2022;13:863088. doi:10.3389/fpsyt.2022.863088

[12] Torre R de la, Farré M, Roset PN, Pizarro N, Abanades S, Segura M, et al. Human Pharmacology of MDMA. *Ther Drug Monit*. 2004;26(2):137-144. doi:10.1097/00007691-200404000-00009

[13] Aguilar MA, García-Pardo MP, Parrott AC. Of mice and men on MDMA: A translational comparison of the neuropsychobiological effects of 3,4-methylenedioxymethamphetamine ('Ecstasy'). *Brain Res*. 2020;1727:146556. doi:10.1016/j.brainres.2019.146556

[14] Mitchell JM, Bogenschutz M, Lilienstein A, Harrison C, Kleiman S, Parker-Guilbert K, et al. MDMA-assisted therapy for severe PTSD: a randomized, double-blind, placebo-controlled phase 3 study. *Nat Med*. 2021;27(6):1025-1033. doi:10.1038/s41591-021-01336-3

[15] PBC M. Investigator's Brochure 2023. Published online March 27, 2023. https://maps.org/wp-content/uploads/2023/04/MDMA-IB-15th-Edition_FINAL_13MAR2023_MRC.pdf. Accessed February 26, 2024

[16] Jerome L, Feduccia AA, Wang JB, Hamilton S, Yazar-Klosinski B, Emerson A, et al. Long-term follow-up outcomes of MDMA-assisted psychotherapy for treatment of PTSD: a longitudinal pooled analysis of six phase 2 trials. *Psychopharmacology*. 2020;237(8):2485-2497. doi:10.1007/s00213-020-05548-2

[17] Mithoefer MC, Feduccia AA, Jerome L, Mithoefer A, Wagner M, Walsh Z, et al. MDMA-assisted psychotherapy for treatment of PTSD: study design and rationale for phase 3 trials based on pooled analysis of six phase 2 randomized controlled trials. *Psychopharmacology*. 2019;236(9):2735-2745. doi:10.1007/s00213-019-05249-5

[18] Nair JB, Hakes L, Yazar-Klosinski B, Paisner K. Fully Validated, Multi-Kilogram cGMP Synthesis of MDMA. *ACS Omega*. 2021;7(1):900-907. doi:10.1021/acsomega.1c05520

[19] Farré M, Abanades S, Roset PN, Peiró AM, Torrens M, O'Mathúna B, et al. Pharmacological Interaction between 3,4-Methylenedioxymethamphetamine (Ecstasy) and Paroxetine: Pharmacological Effects and Pharmacokinetics. *J Pharmacol Exp Ther*. 2007;323(3):954-962. doi:10.1124/jpet.107.129056

[20] Papaseit E, Pérez-Mañá C, Torrens M, Farré A, Poyatos L, Hladun O, et al. MDMA interactions with pharmaceuticals and drugs of abuse. *Expert Opin Drug Metab Toxicol*. 2020;16(5):357-369. doi:10.1080/17425255.2020.1749262

[21] Sarparast A, Thomas K, Malcolm B, Stauffer CS. Drug-drug interactions between psychiatric medications and MDMA or psilocybin: a systematic review. *Psychopharmacology*. 2022;239(6):1945-1976. doi:10.1007/s00213-022-06083-y

[22] Leonardi ETK, Azmitia EC. MDMA (Ecstasy) Inhibition of MAO Type A and Type B: Comparisons with Fenfluramine and Fluoxetine (Prozac). *Neuropsychopharmacol*. 1994;10(4):231-238. doi:10.1038/npp.1994.26

[23] Steuer AE, Boxler MI, Stock L, Kraemer T. Inhibition potential of 3,4-methylenedioxymethamphetamine (MDMA) and its metabolites on the in vitro monoamine oxidase (MAO)-catalyzed deamination of the neurotransmitters serotonin and dopamine. *Toxicol Lett*. 2016;243:48-55. doi:10.1016/j.toxlet.2015.12.001

[24] Popova D, Forsblad A, Hashemian S, Jacobsson SOP. Non-Serotonergic Neurotoxicity by MDMA (Ecstasy) in Neurons Derived from Mouse P19 Embryonal Carcinoma Cells. *Plos One*. 2016;11(11):e0166750. doi:10.1371/journal.pone.0166750

[25] Kaur H, Karabulut S, Gauld JW, Fagot SA, Holloway KN, Shaw HE, et al. Balancing Therapeutic Efficacy and Safety of MDMA and Novel MDXX Analogues as Novel Treatments for Autism Spectrum Disorder. *Psychedelic Med*. 2023;1(3):166-185. doi:10.1089/psymed.2023.0023

[26] Pilgrim JL, Gerostamoulos D, Woodford N, Drummer OH. Serotonin toxicity involving MDMA (ecstasy) and moclobemide. *Forensic Sci Int*. 2012;215(1-3):184-188. doi:10.1016/j.forsciint.2011.04.008

[27] Vuori E, Henry JA, Ojanperä I, Nieminen R, Savolainen T, Wahlsten P, et al. Death following ingestion of MDMA (ecstasy) and moclobemide. *Addiction*. 2003;98(3):365-368. doi:10.1046/j.1360-0443.2003.00292.x

[28] Malcolm B, Thomas K. Serotonin toxicity of serotonergic psychedelics. *Psychopharmacology*. 2022;239(6):1881-1891. doi:10.1007/s00213-021-05876-x

[29] Gudelsky GA, Yamamoto BK. Actions of 3,4-methylenedioxymethamphetamine (MDMA) on cerebral dopaminergic, serotonergic and cholinergic neurons. *Pharmacol Biochem Behav*. 2008;90(2):198-207. doi:10.1016/j.pbb.2007.10.003

[30] Webster JI, Harper DN, Schenk S. Generalization of serotonin and dopamine ligands to the discriminative stimulus effects of different doses of ±3,4-methylenedioxymethamphetamine. *Behav Pharmacol*. 2017;28(4):245-254. doi:10.1097/fbp.0000000000000282

[31] Belmer A, Quentin E, Diaz SL, Guiard BP, Fernandez SP, Doly S, et al. Positive regulation of raphe serotonin neurons by serotonin 2B receptors. *Neuropsychopharmacology*. 2018;43(7):1623-1632. doi:10.1038/s41386-018-0013-0

[32] Llabrés S, García-Ratés S, Cristóbal-Lecina E, Riera A, Borrell JI, Camarasa J, et al. Molecular basis of the selective binding of MDMA enantiomers to the alpha4beta2 nicotinic receptor subtype: Synthesis, pharmacological evaluation and mechanistic studies. *Eur J Med Chem*. 2014;81:35-46. doi:10.1016/j.ejmech.2014.04.044

[33] Paula VF de, Ribeiro A, Pinheiro ML, Sakai M, Lacava MCR, Lapachinske SF, et al. Methylenedioxymethamphetamine (Ecstasy) Decreases Neutrophil Activity and Alters Leukocyte Distribution in Bone Marrow, Spleen and Blood. *Neuroimmunomodulation*. 2009;16(3):191-200. doi:10.1159/000204233

[34] Murnane KS, Kimmel HL, Rice KC, Howell LL. The neuropharmacology of prolactin secretion elicited by 3,4-methylenedioxymethamphetamine ("ecstasy"): A concurrent microdialysis and plasma analysis study. *Horm Behav*. 2012;61(2):181-190. doi:10.1016/j.yhbeh.2011.10.012

[35] Fantegrossi WE, Murai N, Mathúna BÓ, Pizarro N, Torre R de la. Discriminative Stimulus Effects of 3,4-Methylenedioxymethamphetamine and Its Enantiomers in Mice: Pharmacokinetic Considerations. *J Pharmacol Exp Ther*. 2009;329(3):1006-1015. doi:10.1124/jpet.109.150573

[36] Russell LN, Hyatt WS, Gannon BM, Simecka CM, Randolph MM, Fantegrossi WE. Effects of Laboratory Housing Conditions on Core Temperature and Locomotor Activity in Mice. *J Am Assoc Lab Anim Sci : JAALAS*. 2021;60(3):272-280. doi:10.30802/aalas-jaalas-20-000093

[37] Berquist MD, Leth-Petersen S, Kristensen JL, Fantegrossi WE. Locomotor effects of 3,4-methylenedioxymethamphetamine (MDMA) and its deuterated form in mice: psychostimulant effects, stereotypy, and sensitization. *Psychopharmacology*. 2020;237(2):431-442. doi:10.1007/s00213-019-05380-3

[38] Crean RD, Davis SA, Huben SNV, Lay CC, Katner SN, Taffe MA. Effects of (±)3,4-methylenedioxymethamphetamine, (±)3,4-methylenedioxyamphetamine and methamphetamine on temperature and activity in rhesus macaques. *Neuroscience*. 2006;142(2):515-525. doi:10.1016/j.neuroscience.2006.06.033

[39] Morley KC, Arnold JC, McGregor IS. Serotonin (1A) receptor involvement in acute 3,4-methylenedioxymethamphetamine (MDMA) facilitation of social interaction in the rat. *Prog Neuro-psychopharmacology Biological Psychiatry*. 2005;29(5):648-657. doi:10.1016/j.pnpbp.2005.04.009

[40] Morley KC, McGregor IS. (±)-3,4-Methylenedioxymethamphetamine (MDMA, 'Ecstasy') increases social interaction in rats. *Eur J Pharmacol*. 2000;408(1):41-49. doi:10.1016/s0014-2999(00)00749-4

[41] Navarro JF, Rivera A, Maldonado E, Cavas M, Calle A de la. Anxiogenic-like activity of 3,4-methylenedioxy-methamphetamine ("Ecstasy") in the social interaction test is accompanied by an increase of c-fos expression in mice amygdala. *Prog Neuro-Psychopharmacol Biol Psychiatry*. 2004;28(2):249-254. doi:10.1016/j.pnpbp.2003.10.016

[42] Pitts EG, Curry DW, Hampshire KN, Young MB, Howell LL. (±)-MDMA and its enantiomers: potential therapeutic advantages of R(−)-MDMA. *Psychopharmacology*. 2018;235(2):377-392. doi:10.1007/s00213-017-4812-5

[43] Goni-Allo B, Mathúna BÓ, Segura M, Puerta E, Lasheras B, Torre R de la, et al. The relationship between core body temperature and 3,4-methylenedioxymethamphetamine metabolism in rats: implications for neurotoxicity. *Psychopharmacology*. 2008;197(2):263-278. doi:10.1007/s00213-007-1027-1

[44] Baumann MH, Zolkowska D, Kim I, Scheidweiler KB, Rothman RB, Huestis MA. Effects of Dose and Route of Administration on Pharmacokinetics of (±)-3,4-Methylenedioxymethamphetamine in the Rat. *Drug Metab Dispos*. 2009;37(11):2163-2170. doi:10.1124/dmd.109.028506

[45] Fonseca DA, Ribeiro DM, Tapadas M, Cotrim MD. Ecstasy (3,4-methylenedioxymethamphetamine): Cardiovascular effects and mechanisms. *Eur J Pharmacol*. 2021;903:174156. doi:10.1016/j.ejphar.2021.174156

[46] Schmid Y, Rickli A, Schaffner A, Duthaler U, Grouzmann E, Hysek CM, et al. Interactions between Bupropion and 3,4-Methylenedioxymethamphetamine in Healthy Subjects. *J Pharmacol Exp Ther*. 2015;353(1):102-111. doi:10.1124/jpet.114.222356

[47] Hysek CM, Simmler LD, Nicola VG, Vischer N, Donzelli M, Krähenbühl S, et al. Duloxetine Inhibits Effects of MDMA ("Ecstasy") In Vitro and in Humans in a Randomized Placebo-Controlled Laboratory Study. *Plos One*. 2012;7(5):e36476. doi:10.1371/journal.pone.0036476

[48] Frith CH, Chang LW, Lattin DL, Walls RC, Hamm J, Doblin R. Toxicity of methylenedioxymethamphetamine (MDMA) in the dog and the rat. *Fundam Appl Toxicol*. 1987;9(1):110-119. doi:10.1016/0272-0590(87)90158-8

[49] Gonzalez AA, Salinas-Parra N, Cifuentes-Araneda F, Reyes-Martinez C. Vasopressin actions in the kidney renin angiotensin system and its role in hypertension and renal disease. *Vitam Horm*. 2019;113:217-238. doi:10.1016/bs.vh.2019.09.003

[50] Vanattou-Saïfoudine N, McNamara R, Harkin A. Caffeine promotes dopamine D1 receptor-mediated body temperature, heart rate and behavioural responses to MDMA ('ecstasy'). *Psychopharmacology*. 2010;211(1):15-25. doi:10.1007/s00213-010-1864-1

[51] McNamara R, Maginn M, Harkin A. Caffeine induces a profound and persistent tachycardia in response to MDMA ("Ecstasy") administration. *Eur J Pharmacol*. 2007;555(2-3):194-198. doi:10.1016/j.ejphar.2006.10.063

[52] McNamara R, Kerans A, O'Neill B, Harkin A. Caffeine promotes hyperthermia and serotonergic loss following co-administration of the substituted amphetamines, MDMA ("Ecstasy") and MDA ("Love"). *Neuropharmacology*. 2006;50(1):69-80. doi:10.1016/j.neuropharm.2005.08.006

[53] Mueller M, Peters FT, Maurer HH, McCann UD, Ricaurte GA. Nonlinear Pharmacokinetics of (±)3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy") and Its Major Metabolites in

Squirrel Monkeys at Plasma Concentrations of MDMA That Develop After Typical Psychoactive Doses. *J Pharmacol Exp Ther*. 2008;327(1):38-44. doi:10.1124/jpet.108.141366

[54] Mueller M, Goodwin AK, Ator NA, McCann UD, Ricaurte GA. Metabolism and Disposition of 3,4-Methylenedioxymethamphetamine ("Ecstasy") in Baboons after Oral Administration: Comparison with Humans Reveals Marked Differences. *J Pharmacol Exp Ther*. 2011;338(1):310-317. doi:10.1124/jpet.111.180612

[55] Letter EAD, Paepe PD, Clauwaert KM, Belpaire FM, Lambert WE, Bocxlaer JFV, et al. Is vitreous humour useful for the interpretation of 3,4-methylenedioxymethamphetamine (MDMA) blood levels? *Int J Leg Med*. 2000;114(1-2):29-35. doi:10.1007/s004140000135

[56] Scheidweiler KB, Ladenheim B, Barnes AJ, Cadet JL, Huestis MA. (±)-3,4-Methylenedioxymethamphetamine and Metabolite Disposition in Plasma and Striatum of Wild-Type and Multidrug Resistance Protein 1a Knock-Out Mice. *J Anal Toxicol*. 2011;35(7):470-480. doi:10.1093/anatox/35.7.470

[57] MAPS, studies M association for psychedelic. *MDMA Investigator's Brochure*.; 2022:253. https://maps.org/wp-content/uploads/2022/03/MDMA-IB-14th-Edition-FINAL-18MAR2022.pdf. Accessed July 1, 2022

[58] Mueller M, Yuan J, McCann UD, Hatzidimitriou G, Ricaurte GA. Single oral doses of (±) 3,4-methylenedioxymethamphetamine ('Ecstasy') produce lasting serotonergic deficits in non-human primates: relationship to plasma drug and metabolite concentrations. *Int J Neuropsychopharmacol*. 2013;16(4):791-801. doi:10.1017/s1461145712000582

[59] Mueller M, Maldonado-Adrian C, Yuan J, McCann UD, Ricaurte GA. Studies of (±)-3,4-Methylenedioxymethamphetamine (MDMA) Metabolism and Disposition in Rats and Mice: Relationship to Neuroprotection and Neurotoxicity Profile. *J Pharmacol Exp Ther*. 2013;344(2):479-488. doi:10.1124/jpet.112.201699

[60] Peiró AM, Farré M, Roset PN, Carbó M, Pujadas M, Torrens M, et al. Human pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) after repeated doses taken 2 h apart. *Psychopharmacology*. 2011;225(4):883-893. doi:10.1007/s00213-012-2894-7

[61] Torre R de la, Yubero-Lahoz S, Pardo-Lozano R, Farré M. MDMA, methamphetamine, and CYP2D6 pharmacogenetics: what is clinically relevant? *Front Genet*. 2012;3:235. doi:10.3389/fgene.2012.00235

[62] Omer VEVSt, Ali SF, Holson RR, Duhart HM, Scalzo FM, Slikker W. Behavioral and neurochemical effects of prenatal methylenedioxymethamphetamine (MDMA) exposure in rats. *Neurotoxicol Teratol*. 1991;13(1):13-20. doi:10.1016/0892-0362(91)90022-o

[63] Schenk S, Gittings D, Johnstone M, Daniela E. Development, maintenance and temporal pattern of self-administration maintained by ecstasy (MDMA) in rats. *Psychopharmacology*. 2003;169(1):21-27. doi:10.1007/s00213-003-1407-0

[64] Oberlender R, Nichols DE. Drug discrimination studies with MDMA and amphetamine. *Psychopharmacology*. 1988;95(1):71-76. doi:10.1007/bf00212770

[65] Gold LH, Koob GF. MDMA produces stimulant-like conditioned locomotor activity. *Psychopharmacology*. 1989;99(3):352-356. doi:10.1007/bf00445556

[66] Fantegrossi W, Winger G, Woods J. INTRAVENOUS SELF-ADMINISTRATION OF MDMA AND ITS STEREOISOMERS BY RHESUS MONKEYS. *Behav Pharmacol*. 1999;10(SUPPLEMENT 1):s33. doi:10.1097/00008877-199908001-00081

[67] Fantegrossi WE. Reinforcing effects of methylenedioxy amphetamine congeners in rhesus monkeys: are intravenous self-administration experiments relevant to MDMA neurotoxicity? *Psychopharmacology*. 2005;189(4):471-482. doi:10.1007/s00213-006-0320-8

[68] Robledo P, Balerio G, Berrendero F, Maldonado R. Study of the behavioural responses related to the potential addictive properties of MDMA in mice. *Naunyn-Schmiedeberg's Arch Pharmacol*. 2004;369(3):338-349. doi:10.1007/s00210-003-0862-9

[69] Smith DA, Blough BE, Banks ML. Cocaine-like discriminative stimulus effects of amphetamine, cathinone, methamphetamine, and their 3,4-methylenedioxy analogs in male rhesus monkeys. *Psychopharmacology*. 2017;234(1):117-127. doi:10.1007/s00213-016-4444-1

[70] Rosas-Hernandez H, Cuevas E, Lantz SM, Rice KC, Gannon BM, Fantegrossi WE, et al. Methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxypyrovalerone (MDPV) induce differential cytotoxic effects in bovine brain microvessel endothelial cells. *Neurosci Lett*. 2016;629:125-130. doi:10.1016/j.neulet.2016.06.029

[71] Maples-Keller JL, Norrholm SD, Burton M, Reiff C, Coghlan C, Jovanovic T, et al. A randomized controlled trial of 3,4-methylenedioxymethamphetamine (MDMA) and fear extinction retention in healthy adults. *J Psychopharmacol*. 2022;36(3):368-377. doi:10.1177/02698811211069124

[72] Therapeutics L. Midomafetamine (MDMA) Capsule with psychological intervention (MDMA-AT) . Published online June 4, 2024. https://www.fda.gov/media/178986/download. Accessed February 26, 2025

[73] JAY N, BERRA YK, JULIAN N, ARUL B, LINDA H, JOHN B. COMPOSITIONS COMPRISING MDMA AND METHODS OF USING SAME. Published online 2024.

[74] Huestis MA, Smith WB, Leonowens C, Blanchard R, Viaccoz A, Spargo E, et al. MDMA pharmacokinetics: A population and physiologically based pharmacokinetics model-informed analysis. *CPT: Pharmacomet Syst Pharmacol*. 2025;14(2):376-388. doi:10.1002/psp4.13282

[75] Bershad AK, Hsu DT, Wit H de. MDMA enhances positive affective responses to social feedback. *J Psychopharmacol*. 2024;38(3):297-304. doi:10.1177/02698811231224153

[76] Straumann I, Avedisian I, Klaiber A, Varghese N, Eckert A, Rudin D, et al. Acute effects of R-MDMA, S-MDMA, and racemic MDMA in a randomized double-blind cross-over trial in healthy participants. *Neuropsychopharmacology*. 2025;50(2):362-371. doi:10.1038/s41386-024-01972-6

[77] Danforth AL, Struble CM, Yazar-Klosinski B, Grob CS. MDMA-assisted therapy: A new treatment model for social anxiety in autistic adults. *Prog Neuro-Psychopharmacol Biol Psychiatry*. 2016;64:237-249. doi:10.1016/j.pnpbp.2015.03.011

[78] Wolfson PE, Andries J, Feduccia AA, Jerome L, Wang JB, Williams E, et al. MDMA-assisted psychotherapy for treatment of anxiety and other psychological distress related to life-threatening illnesses: a randomized pilot study. *Sci Rep*. 2020;10(1):20442. doi:10.1038/s41598-020-75706-1

[79] O'Mathúna B, Farré M, Rostami-Hodjegan A, Yang J, Cuyàs E, Torrens M, et al. The Consequences of 3,4-Methylenedioxymethamphetamine Induced CYP2D6 Inhibition in Humans. *J Clin Psychopharmacol*. 2008;28(5):523-529. doi:10.1097/jcp.0b013e318184ff6e

[80] Yubero-Lahoz S, Pardo R, Farré M, O'Mahony B, Torrens M, Mustata C, et al. Sex Differences in 3,4-Methylenedioxymethamphetamine (MDMA; Ecstasy)-Induced Cytochrome P450 2D6 Inhibition in Humans. *Clin Pharmacokinet*. 2011;50(5):319-329. doi:10.2165/11584550-000000000-00000

[81] Vizeli P, Schmid Y, Prestin K, Schwabedissen HEM zu, Liechti ME. Pharmacogenetics of ecstasy: CYP1A2, CYP2C19, and CYP2B6 polymorphisms moderate pharmacokinetics of MDMA in healthy subjects. *Eur Neuropsychopharmacol*. 2017;27(3):232-238. doi:10.1016/j.euroneuro.2017.01.008

[82] Vizeli P, Liechti ME. Safety pharmacology of acute MDMA administration in healthy subjects. *J Psychopharmacol*. 2017;31(5):576-588. doi:10.1177/0269881117691569

[83] Hysek C, Schmid Y, Rickli A, Simmler L, Donzelli M, Grouzmann E, et al. Carvedilol inhibits the cardiostimulant and thermogenic effects of MDMA in humans. *Br J Pharmacol*. 2012;166(8):2277-2288. doi:10.1111/j.1476-5381.2012.01936.x

[84] Sprague JE, Moze P, Caden D, Rusyniak DE, Holmes C, Goldstein DS, et al. Carvedilol reverses hyperthermia and attenuates rhabdomyolysis induced by 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) in an animal model* *Crit Care Med*. 2005;33(6):1311-1316. doi:10.1097/01.ccm.0000165969.29002.70

[85] Perna EB de SF, Theunissen EL, Kuypers KPC, Heckman P, Torre R de la, Farre M, et al. Memory and mood during MDMA intoxication, with and without memantine pretreatment. *Neuropharmacology*. 2014;87:198-205. doi:10.1016/j.neuropharm.2014.03.008

[86] Liechti ME, Vollenweider FX. Acute psychological and physiological effects of MDMA ("Ecstasy") after haloperidol pretreatment in healthy humans. *Eur Neuropsychopharm*. 2000;10(4):289-295. doi:10.1016/s0924-977x(00)00086-9

[87] Gamma A, Buck A, Berthold T, Liechti ME, Vollenweider FX, Hell D. 3,4-Methylenedioxymethamphetamine (MDMA) Modulates Cortical and Limbic Brain Activity as Measured by [H215O]-PET in Healthy Humans. *Neuropsychopharmacol*. 2000;23(4):388-395. doi:10.1016/s0893-133x(00)00130-5

[88] Singleton SP, Wang JB, Mithoefer M, Hanlon C, George MS, Mithoefer A, et al. Altered brain activity and functional connectivity after MDMA-assisted therapy for post-traumatic stress disorder. *Front Psychiatry*. 2023;13:947622. doi:10.3389/fpsyt.2022.947622

[89] Grob CS, Poland RE, Chang L, Ernst T. Psychobiologic effects of 3,4-methylenedioxymethamphetamine in humans: methodological considerations and preliminary observations. *Behav Brain Res*. 1995;73(1-2):103-107. doi:10.1016/0166-4328(96)00078-2

[90] Liechti ME, Gamma A, Vollenweider FX. Gender differences in the subjective effects of MDMA. *Psychopharmacology*. 2001;154(2):161-168. doi:10.1007/s002130000648

[91] Freedman RR, Johanson CE, Tancer ME. Thermoregulatory effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans. *Psychopharmacology*. 2005;183(2):248-256. doi:10.1007/s00213-005-0149-6

[92] Kolk BA van der, Wang JB, Yehuda R, Bedrosian L, Coker AR, Harrison C, et al. Effects of MDMA-assisted therapy for PTSD on self-experience. *PLOS ONE*. 2024;19(1):e0295926. doi:10.1371/journal.pone.0295926

[93] Nicholas CR, Wang JB, Coker A, Mitchell J, Klaire S, Yazar-Klosinki B, et al. The Effects of MDMA-Assisted Therapy on Alcohol and Substance Use in a Phase 3 Trial for Treatment of Severe PTSD. *Drug Alcohol Depen*. Published online 2022:109356. doi:10.1016/j.drugalcdep.2022.109356

[94] Brewerton TD, Wang JB, Lafrance A, Pamplin C, Mithoefer M, Yazar-Klosinki B, et al. MDMA-assisted therapy significantly reduces eating disorder symptoms in a randomized placebo-controlled trial of adults with severe PTSD. *J Psychiatr Res*. 2022;149:128-135. doi:10.1016/j.jpsychires.2022.03.008

[95] Yang J, Wang N, Luo W, Gao J. The efficacy and safety of MDMA-assisted psychotherapy for treatment of posttraumatic stress disorder: A systematic review and meta-analysis from randomized controlled trials. *Psychiatry Res*. 2024;339:116043. doi:10.1016/j.psychres.2024.116043

[96] Bouso JC, Doblin R, Farré M, Alcázar MÁ, Gómez-Jarabo G. MDMA-Assisted Psychotherapy Using Low Doses in a Small Sample of Women with Chronic Posttraumatic Stress Disorder. *J Psychoact Drugs*. 2008;40(3):225-236. doi:10.1080/02791072.2008.10400637

[97] Colcott J, Guerin AA, Carter O, Meikle S, Bedi G. Side-effects of mdma-assisted psychotherapy: a systematic review and meta-analysis. *Neuropsychopharmacology*. 2024;49(8):1208-1226. doi:10.1038/s41386-024-01865-8

[98] Murray CH. Common side effects of MDMA-assisted psychotherapy. *Neuropsychopharmacology*. 2024;49(8):1206-1207. doi:10.1038/s41386-024-01873-8

[99] Simonsson O, Johnson MW, Hendricks PS. Psychedelic and MDMA-Related Adverse Effects—A Call for Action. *JAMA Heal Forum*. 2024;5(11):e243630. doi:10.1001/jamahealthforum.2024.3630

[100] Atila C, Straumann I, Vizeli P, Beck J, Monnerat S, Holze F, et al. Oxytocin and the Role of Fluid Restriction in MDMA-Induced Hyponatremia. *JAMA Netw Open*. 2024;7(11):e2445278. doi:10.1001/jamanetworkopen.2024.45278

[101] Lykos. Lykos Therapeutics Announces FDA Acceptance and Priority Review of New Drug Application for MDMA-Assisted Therapy for PTSD - Feb 9, 2024. https://news.lykospbc.com/2024-02-09-Lykos-Therapeutics-Announces-FDA-Acceptance-and-Priority-Review-of-New-Drug-Application-for-MDMA-Assisted-Therapy-for-PTSD. Accessed February 29, 2024

[102] Lykos. MAPS Public Benefit Corporation Announces Oversubscribed Series A Financing and Renames to Lykos Therapeutics - Lykos. Published 2024. https://lykospbc.com/press-releases/maps-public-benefit-corporation-announces-oversubscribed-series-a-financing-and-renames-to-lykos-therapeutics/