

**3,4-Methylenedioxyamphetamine**  
**(MDMA, “Ecstasy”)**

**Studies on MDMA Metabolism and Pharmacokinetics in  
Humans and Squirrel Monkeys**

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# 1 GENERAL PART

## 1.1 INTRODUCTION

Drug abuse is a widespread problem in societies all over the world. Especially, so-called designer drugs are increasingly popular among young people. The most frequently abused drugs are amphetamine (AM), methamphetamine and their derivatives, such as 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") (1). The AM-derived designer drugs MDMA ("Ecstasy" or "Adam") and its demethylated analogue MDA ("Love Pills" or "Eve"), also a metabolite of MDMA, are psychotropic agents chemically and pharmacologically related to AM and mescaline. MDMA was first synthesized in 1912 as a chemical intermediate for the vasoconstrictor hydrastinine. In contrast to the popular believe that MDMA was developed as an appetite suppressant, it was actually patented without any specific purpose by Merck in 1914 (2). MDA on the other hand was patented for different uses such as cough suppressant or appetite inhibitor, but was never marketed (3). In the late seventies MDMA was discovered by the psychedelic therapy community as catalyst to psychotherapy. It was described as mind-loosening and to facilitate interpersonal communication and intimacy. For that reason Nichols coined the term "entactogen" which means "to produce a touching within" (4, 5). In the early eighties the non-medical use of MDMA and MDA gained great popularity as so called "rave drug" because of their common use at dance clubs or large, organized dance parties ("raves"). In Europe and North America designer drugs are sold in a variety of colored and professional-looking tablets, stamped with different symbols according to the ideas of the producer. Some examples are presented in Fig. 1 (3). However, purity and identity of such pills sold as MDMA might be doubtful (6, 7). Because of the permanently increasing consumption and the suspicion of neurotoxic effects MDMA and MDA were placed in Schedule 1 of the restricted drugs list in United States in 1985. Shortly thereafter the European countries followed this example (3, 5). Despite the fact that MDMA has caused harm and death, its potential to destroy brain serotonin (5-HT) axon terminals (8-10), and its lack of recognized therapeutic potential, the Multidisciplinary Association for Psychedelic Studies (MAPS) lobbies intensively trying to legalize MDMA for research purposes (4, 5). As a result of their efforts currently four clinical trials are underway (clinicaltrials.gov identifiers NCT00252174, NCT00090064, NCT00402298,

and NCT00353938). At least in part, MDMA use and abuse continues because implications of the neurotoxic effects in animals for humans are uncertain. This uncertainty stems from discrepancies in dosing, route of administration and pharmacokinetic parameters between animal studies and human MDMA use (11, 12).



Fig. 1: "Ecstasy" tablets sold on the illicit drug market

### 1.1.1 Chemistry

Chemically, MDMA and MDA are designated as *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane and 1-(3,4-methylenedioxyphenyl)-2-aminopropane, respectively. According nomenclature of the International Union of Pure and Applied Chemistry (IUPAC) MDMA is characterized as 1-benzo[1,3]dioxol-5-yl-*N*-methyl-propan-2-amine and MDA as 1-benzo[1,3]dioxol-5-yl-propan-2-amine. Structurally they are related to the psychomotor stimulant AM and the hallucinogen mescaline. MDMA and MDA are chiral compounds because they each contain an asymmetric carbon atom in the side chain. Consequently they exist as pairs of optical isomers. According to the Cahn-Ingold-Prelog convention, the levorotatory enantiomers carry the *R*-configuration and the dextrorotatory enantiomers the *S*-configuration (8).

Their chemical structures are shown in Fig. 2. On the illicit market the amphetamine-derived designer drugs are sold as racemates synthesized from achiral educts by non-enantioselective procedures, e.g. piperonal or 3,4-methylenedioxyphenylacetone (13, 14).

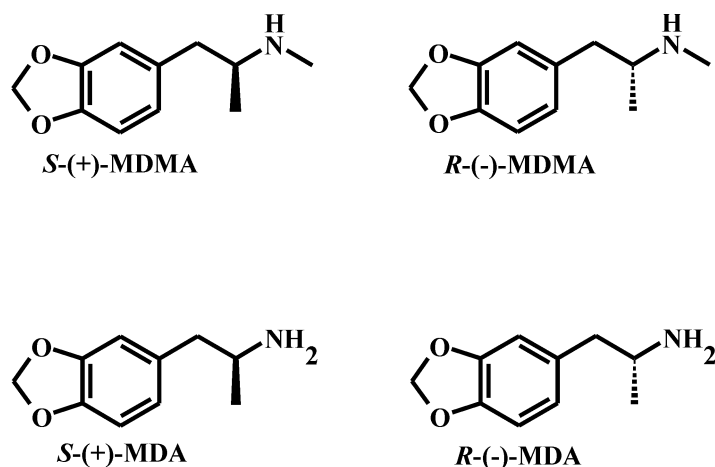


Fig. 2: Chemical structures of the enantiomers of MDMA and MDA.

### 1.1.2 Pharmacodynamics and acute toxicity

The extensively studied mechanism of action of the AM-derived designer drugs is mainly based on their effects on the presynaptic terminal of serotonergic, noradrenergic, and, to a lesser degree, dopaminergic neurons (3, 8, 15). MDMA and MDA are so called indirect agents because they exert their effects primarily through release of 5-HT rather than by direct actions on 5-HT receptors. Following, the detailed mechanism of action is shown on the example of a 5-HT neuron. MDMA and MDA present high affinity on presynaptic 5-HT uptake transporters (SERT) located in the nerve terminals. MDMA and MDA are co-transported with sodium ions ( $\text{Na}^+$ ) into the terminal via SERT, competitively inhibiting 5-HT uptake. Once inside the cell, MDMA and MDA are carried into the storage vesicles by the vesicular monoamine transporter (VMAT) and 5-HT is released in exchange. By this mechanism cytoplasmatic 5-HT and  $\text{Na}^+$  levels rise. Furthermore, cellular 5-HT increase is caused by inhibition of the monoamine oxidase (MAO) which normally mediates 5-HT degradation. 5-HT binds to the now inward-facing transporter and, together with  $\text{Na}^+$ , is transported out of the terminal into the synaptic

cleft where it activates the postsynaptic receptors (15). Additionally, 5-HT is released due to the antagonistic effect of MDMA on  $\alpha_2$ -adrenoceptors on brain sites (16).

Apart from these mechanisms, MDMA shows agonistic effects on postsynaptic 5-HT<sub>2</sub> receptors and M<sub>1</sub>-muscarinic receptors and mediates inhibition of 5-HT synthesis by decreasing the tryptophan hydroxylase activity (16, 17).

The amphetamine-derived designer drugs exhibit stimulation of the central nervous system mainly due to their enhancement of serotonergic neurotransmission. The desired mental effects include euphoria, well-being, sharpened sensory perception, greater sociability, heightened sense of closeness to other people, and greater tolerance of their views and feelings (3, 8, 14). Increased noradrenalin levels are responsible for physical effects such as sense of energy, appetite loss, and sexual arousal.

However, MDMA and MDA abuse is not without risk. Psychological signs of intoxications are agitation, hallucinations, panic disorder, paranoid psychosis, depression and anxiety. Acute physical side effects include tachycardia, hypertension, increased muscle tension, bruxism, sweating, hyperpyrexia, nausea, blurred vision, and ataxia (3-5, 8, 13). Many severe or even fatal intoxications have been described (3, 5, 8, 13). MDMA-related deaths are generally caused by hyperpyrexia followed by rhabdomyolysis with disseminated intravascular coagulation and multi organ failure, 5-HT syndrome along with hyperthermia or dilutional hyponatraemia together with cerebral edema (18).

### **1.1.3 Pharmacokinetics**

After oral administration MDMA is rapidly absorbed from the intestinal tract with plasma concentrations peaking about two hours after ingestion (3, 19). The lipophilic drug passes readily into various tissues and accumulates or binds to tissue constituents (3, 20, 21). MDMA plasma elimination half-life is about eight hours (3, 19, 22). Urinary recovery of the parent compound represents about 15% which indicates that the drug is mainly eliminated by metabolism (22). MDMA metabolism is mediated by several different enzymes [for example enzymes from the cytochrome P450 family (CYP) and

catechol-O-methyl transferase (COMT)] and proceeds via two major pathways which operate in unison but at different rates (Fig. 3) (22-25). The first pathway, predominant in humans, involves O-demethylenation to 3,4-dihydroxymethamphetamine (HHMA) followed by O-methylation to 4-hydroxy-3-methoxymethamphetamine (HMMA) and O-conjugation with sulfate or glucuronic acid. The second entails initial N-demethylation to MDA, followed by deamination and oxidation to the corresponding benzoic acid derivatives conjugated with glycine (26, 27). Alternatively, MDA also undergoes O-demethylenation to 3,4-dihydroxyamphetamine (HHA) following O-methylation to 4-hydroxy-3-methoxyamphetamine (HMA) and O-conjugation with glucuronic acid or sulfate (see above). HHMA and HMMA present the main metabolites in plasma, whereas MDA appears to be only a minor metabolite, accounting for less than 5% of the MDMA concentrations found in plasma (19, 22). The catechols HHMA and HHA can easily be oxidized to their corresponding ortho-quinones which in turn can form adducts with glutathione and other thiol-containing compounds (28, 29).

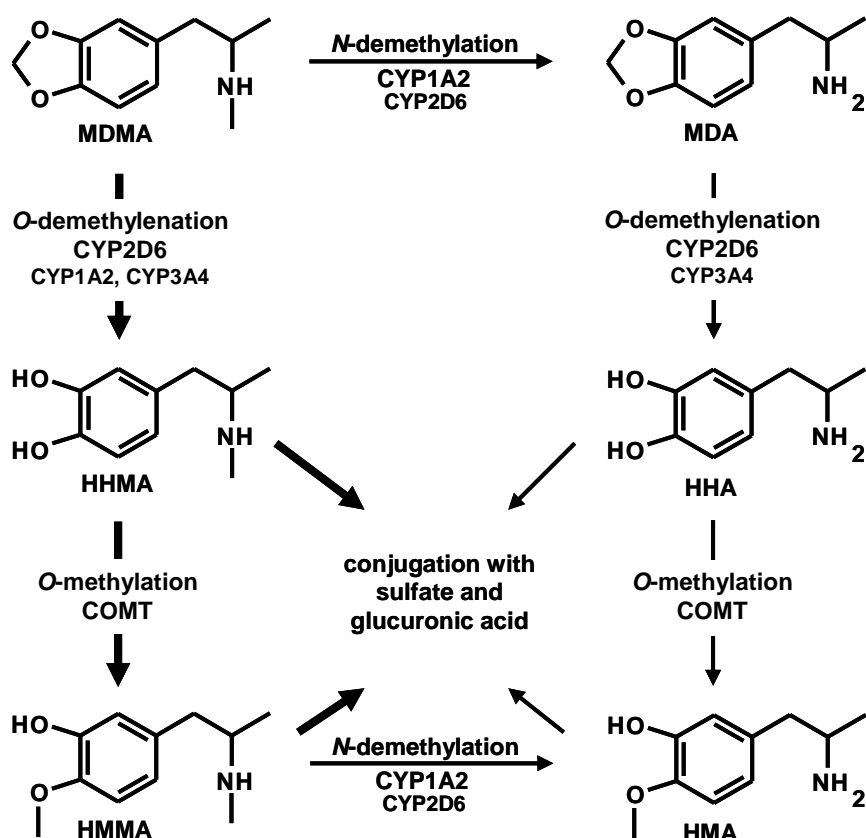


Fig. 3: MDMA metabolism including the enzymes mainly involved in these conversions. In humans ring-demethylenation predominates, whereas in rodents N-demethylenation is more prominent.

Interestingly, MDMA pharmacokinetics has been postulated to be nonlinear (19, 30). In detail, MDMA plasma levels increase disproportionately with dose. This phenomenon could have significant impact on public health if adverse effects are related to the parent compound, especially since nonlinearity already occurs at plasma levels that are typical after human MDMA doses (1 – 2 mg/kg) (19, 22). Mechanism-based inhibition of CYP2D6 by MDMA could be an explanation for nonlinear pharmacokinetics. In vitro data suggest that a metabolic complex formed by the methylenedioxyphenyl ring is responsible for the auto-inhibition of MDMA metabolism (31).

#### **1.1.4 MDMA brain neurotoxicity**

A large body of preclinical research data demonstrates that MDMA has the potential to destroy brain 5-HT axon terminals (8-10). Long-lasting depletion of 5-HT in the central nervous system, persistent depression of tryptophan hydroxylase activity, and reduction of the density of 5-HT uptake sites and VMAT provide evidence for neurotoxicity. Additionally, after MDMA treatment argyrophillic cells can be found in 5-HT regions. Axon swelling and fragmentation in the short term followed by decreased immunoreactivity in the long term indicate cell death as result of necrosis (32).

The brain 5-HT neurotoxicity evokes a number of neuropsychiatric sequelae named in the following. Cognitive deficits (e.g. impaired visual and verbal memory) correlating with the loss of SERT (33, 34), alteration in circadian activity, changed sleep patterns (35), endocrine dysfunctions, impulsivity (36), and mood disorders, such as anxiety and depression (37).

Despite much research, the precise mechanism by which MDMA produces 5-HT neurotoxic effects has yet to be identified. Several mechanisms are proposed: Generation of a toxic drug metabolite (catecholic or trihydroxy derivatives, namely HHMA and 2,4,5-trihydroxymethamphetamine) or an endogenous neurotransmitter metabolite (6-hydroxydopamine, 5,6-dihydroxytryptamine, or 5,7-dihydroxytryptamine) which in turn causes oxidative stress and radical-mediated cell damage (32). There is growing interest in the possible role of neurotoxic thioether conjugates, formed by the corresponding quinones of HHMA or HHA and glutathione or acetylcysteine, with subsequent protein denaturation and lipid peroxidation of the cell membranes (28, 38-



41). On the other hand, extensive series of pharmacological and toxicological studies have suggested that brain dopamine (DA) mediates MDMA neurotoxicity by formation of free radicals after DA uptake into 5-HT presynapses and consequent degradation by MAO (42-44). Furthermore, non-enzymatic transformation of tyrosine also has been implicated in causing 5-HT neurotoxicity (45, 46). Cell death due to destabilization of calcium homeostasis (a result of continued glutamate mediated 5-HT neuron excitation) could also be responsible for the neurotoxic effects (32, 47). At last but not least, temperature seems to play an important role in MDMA mediated brain neurotoxicity. Increasing body temperature has been postulated to lead to blood brain barrier disruption, brain edema and cell injury (48). Additionally, glycogen depletion with subsequent cell death due to increased brain glucogenolysis as a result of inadequate energy supply has been linked to MDMA caused hyperthermia (49, 50).

Only few publications are available on the neurotoxicity in living humans. In these studies, recreational MDMA users were found to have decreased levels of 5-hydroxyindoleacetic acid, the main metabolite of 5-HT, in the cerebrospinal fluid (51) and a reduced density of 5-HT transporters in the brain as determined by positron emission computed tomography with a ligand selective for these transporters (52, 53). Both findings are indicative of 5-HT neurotoxicity in humans. Unfortunately, these studies were performed with recreational users, so it cannot be excluded whether the reported findings might also be due to use of other recreational drugs especially since polydrug use is not uncommon. As studies involving controlled administration of MDMA are problematic for ethical reasons, animal studies are a good possibility for systematically studying the neurotoxicity of these drugs. However, results from animal studies are not always transferable on humans. The toxic effects and pharmacokinetics can differ considerably between humans and different animal species. Moreover, despite a large body of preclinical research, demonstrating that MDMA has the potential to destroy 5-HT axon terminals, MDMA use and abuse continue (8-10). At least in part, MDMA use continues because the relevance of much of the animal MDMA neurotoxicity data to humans is uncertain. This uncertainty stems from the fact that the majority of animal studies have used multiple high doses, have given these doses systemically rather than orally (as mainly taken by humans) and, most often, have used rodents (rats and mice), which metabolize MDMA differently than primates (54). Detailed information on the pharmacokinetics of MDMA in different animal species and their comparison to

the pharmacokinetics in humans is important to estimate the transferability of neurotoxicity studies from animal models on humans.

## **1.2 AIMS AND SCOPES**

Determination of pharmacokinetic profiles of oral MDMA doses in animal models might help to bridge the gap between MDMA neurotoxicity studies in animals and human use patterns. Furthermore, by characterizing the formation of various MDMA metabolites in different species, it might be possible to gain insight into mechanisms of MDMA neurotoxicity.

Therefore, the aims of the presented studies were:

- Development of LC-MS procedures for determination of MDMA and its main metabolites in samples from different species (rat, squirrel monkey, human)
- Determination of the metabolic pattern and the pharmacokinetic profile of MDMA in different species
- Comparison of the obtained species-specific data.

## **2 PUBLICATIONS TO THE RESULTS**

The results of the studies were published in the following papers:

**2.1 VALIDATED LIQUID CHROMATOGRAPHIC-ELECTROSPRAY IONIZATION MASS SPECTROMETRIC ASSAY FOR SIMULTANEOUS DETERMINATION OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE AND ITS METABOLITES 3,4-METHYLENEDIOXYAMPHETAMINE, 3,4-DIHYDROXYMETHAMPHETAMINE, AND 4-HYDROXY-3-METHOXYMETHAMPHETAMINE IN SQUIRREL MONKEY PLASMA (55)**

**(DOI: 10.1016/J.JCHROMB.2007.06.034)**



**2.2 HYDROLYSIS OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE  
(MDMA) METABOLITE CONJUGATES IN HUMAN, SQUIRREL MONKEY AND RAT  
PLASMA (56)**

**(DOI: 10.1007/s00216-009-2607-1)**



**2.3 SIMULTANEOUS LIQUID CHROMATOGRAPHIC-ELECTROSPRAY IONIZATION MASS SPECTROMETRIC QUANTIFICATION OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA, ECSTASY) AND ITS METABOLITES 3,4-DIHYDROXYMETHAMPHETAMINE, 4-HYDROXY-3-METHOXYMETHAMPHETAMINE AND 3,4-METHYLENEDIOXYAMPHETAMINE IN SQUIRREL MONKEY AND HUMAN PLASMA AFTER ACIDIC CONJUGATE CLEAVAGE (57)**

**(DOI: 10.1016/J.FORSCIINT.2008.12.002)**





**2.4 NON-LINEAR 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 (58)**

**(DOI: 10.1124/JPET.108.141366)**



**2.5 DIRECT COMPARISON OF ( $\pm$ ) 3, 4-METHYLENEDIOXYMETHAMPHETAMINE  
(MDMA, “*ECSTASY*”) DISPOSITION AND METABOLISM  
IN HUMANS AND SQUIRREL MONKEYS (59)**

**(DOI: 10.1097/FTD.0B013E3181A4F6C2)**



### 3 CONCLUSIONS

Studies on MDMA metabolite formation in different species (rat, squirrel monkey and human) indicated species differences in hydrolysis of MDMA metabolites, which need to be considered in specimen preparation. To maximize recovery of MDMA metabolites in human or squirrel monkey plasma acidic hydrolysis should be utilized, while in rat enzymatic hydrolysis should be employed.

The developed analytical procedures allowing the detection of MDMA and its major metabolites in biological samples of humans, squirrel monkeys, and rats previously treated with MDMA proved useful for acquiring pharmacokinetic data in either species.

Further studies in human and squirrel monkeys showed similar but not identical metabolic pathways. In particular, amounts of HHMA and MDA were comparable, but formation of HMMA was more extensive in squirrel monkeys than humans. The squirrel monkey also revealed a shorter  $T_{1/2}$  of MDMA. In both species, nonlinear pharmacokinetics were firmly established at comparable MDMA plasma levels. Altogether, the squirrel monkey seemed to be an appropriate model for predicting outcomes of MDMA exposure in humans, although this will depend upon the pharmacokinetic parameter of MDMA or its metabolites that mostly influences the outcome of interest. Since nonlinear MDMA accumulation occurred at MDMA plasma levels that develop in humans after taking typical doses, the already small gap between safe and toxic MDMA doses in primates might be more narrow than expected, meaning that small increase in dose could have a huge impact on likelihood and severity of MDMA toxicities, including brain serotonin neurotoxicity.



## 4 SUMMARY

In the presented studies, the pharmacokinetic profile and metabolic pattern of MDMA in different species were determined. Furthermore species-specific differences on conjugate cleavage of the phase II metabolites were investigated in human, squirrel monkey, and rat. After optimization of cleavage conditions respectively for each species, liquid chromatography-mass spectrometry (LC-MS)-based assay procedures were developed and focused on determination of the parent compound and its corresponding major metabolites in plasma of different species. After administration of different oral MDMA doses pharmacokinetics for MDMA and its metabolites (MDA, HHMA, and HMMA) were determined in squirrel monkey and human. In both species nonlinear pharmacokinetics were firmly established with nonlinear MDMA accumulation occurring at plasma MDMA levels that develop in humans after typical doses. Comparison of pharmacokinetics of MDMA and its metabolites between humans and squirrel monkeys revealed the squirrel monkey as appropriate model for predicting outcomes of MDMA exposure in humans depending upon the pharmacokinetic parameter of MDMA or its metabolites that mostly influences the outcome of interest.





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## 6 ZUSAMMENFASSUNG

In dieser Dissertation wurden Metabolismus und Pharmakokinetiken von MDMA in verschiedenen Spezies untersucht. Desweiteren wurden Studien bezüglich unterschiedlicher Entstehung von Phase II Stoffwechselprodukten in Mensch, Totenkopffäffchen und Ratte durchgeführt. Nachdem die Reaktionsbedingungen zur Konjugatspaltung für jede Spezies entsprechend optimiert wurden, konnten Flüssigchromatographie-Massenspektrometrie (LC-MS)-basierte Verfahren zur Quantifizierung von MDMA und seinen Hauptmetaboliten (MDA, HHMA und HMMA) in Plasma von Mensch und Totenkopffaffe entwickelt werden. Nach Behandlung mit verschiedenen Dosierungen von MDMA zeigten Mensch und Affe eine nicht-lineare Pharmakokinetik der Muttersubstanz, und zwar nach Plasmaspiegeln, die beim Menschen bereits nach typischer Ecstasy-Einnahme auftreten. Vergleich der Pharmakokinetiken zwischen beiden Spezies führte zu der Schlussfolgerung, dass der Totenkopffaffe ein geeignetes Tiermodell darstellt, um Aussagen über die Wirkungen von MDMA im Menschen zu treffen. Im Einzelnen hängt die Aussagekraft dieses Tiermodells jedoch davon ab, welcher pharmakokinetischer Parameter von MDMA oder einem seiner Metaboliten die pharmakodynamische Wirkung, die untersucht werden soll, am stärksten beeinflusst.