Urinary Excretion Kinetics of 3,4-Methylenedioxymethamphetamine (MDMA, Ecstasy) and Its Phase I and Phase II Metabolites in Humans following Controlled MDMA Administration

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BACKGROUND: 3,4-Methylenedioxymethamphetamine (MDMA) is excreted in human urine as unchanged drug and phase I and II metabolites. Previous urinary excretion studies after controlled oral MDMA administration have been performed only after conjugate cleavage. Therefore, we investigated intact MDMA glucuronide and sulfate metabolite excretion.

METHODS: We used LC–high-resolution MS and GC-MS to reanalyze blind urine samples from 10 participants receiving 1.0 or 1.6 mg/kg MDMA orally. We determined median $C_{\text{max}}$, $t_{\text{max}}$, first and last detection times, and total urinary recovery; calculated ratios of sulfates and glucuronides; and performed in vitro–in vivo correlations.

RESULTS: Phase II metabolites of 3,4-dihydroxymethamphetamine (DHMA), 4-hydroxy-3-methoxymethamphetamine (HMMA), 3,4-dihydroxyamphetamine (DHA), and 4-hydroxy-3-methoxyamphetamine were identified, although only DHMA sulfates, HMMA sulfate, and HMMA glucuronide had substantial abundance. Good correlation was observed for HMMA measured after acid hydrolysis and the sum of unconjugated HMMA, HMMA glucuronide, and HMMA sulfate ($R^2 = 0.87$). More than 90% of total DHMA and HMMA were excreted as conjugates. The analyte with the longest detection time was HMMA sulfate. Median HMMA sulfate/glucuronide and DHMA 3-sulfate/4-sulfate ratios for the first 24 h were 2.0 and 5.3, respectively, in accordance with previous in vitro calculations from human liver microsomes and cytosol experiments.

CONCLUSIONS: Human MDMA urinary metabolites are primarily sulfates and glucuronides, with sulfates present in higher concentrations than glucuronides. This new knowledge may lead to improvements in urine MDMA and metabolite analysis in clinical and forensic toxicology, particularly for the performance of direct urine analysis.

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Drug Monitoring and Toxicology

Clinical Chemistry 57:12
1748–1756 (2011)

3,4-Methylenedioxymethamphetamine (MDMA),4 known as ecstasy, is a popular recreational drug leading to feelings of euphoria and energy and a desire to socialize (1, 2). In recent publications from the Substance Abuse and Mental Health Services Administration report that MDMA consumption is increasing again in the US (3). MDMA can induce severe acute toxic symptoms such as tachycardia, hypertension, hyperthermia, and hepatotoxicity (2), and many fatal intoxications have been described. Data from animal experiments suggest that chronic toxicity from MDMA causes irreversible damage to serotonergic nerve terminals in the central nervous system at currently administered doses (2, 4–6). In humans, chronic MDMA toxicity is still controversial, because recent publications suggest that the doses administered in animals may be too high compared to those taken by humans (7, 8). Still, data suggest that humans are susceptible to brain serotonin neurotoxicity (9). Direct injection of MDMA into brain fails to reproduce the neurotoxic effects seen after systemic administration (10). Furthermore, alteration of cytochrome P450–mediated

4 Nonstandard abbreviations: MDMA, 3,4-methylenedioxymethamphetamine; DHMA, 3,4-dihydroxymethamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine; DHA, 3,4-dihydroxyamphetamine; UGT, uridine diphosphate glucuronyltransferase; MDA, 3,4-methylenedioxymethamphetamine; pholedrine, 4-hydroxymethamphetamine; NIDA, National Institute on Drug Abuse; LC-HRMS, liquid chromatography/high-resolution mass spectrometry; GC-NICI-MS, gas chromatography/negative-ion chemical ionization mass spectrometry; $C_{\text{max}}$, maximum concentration; $C_{\text{max}100}$, normalized to 100 mg/dL creatinine; $t_{\text{max}}$, time of maximum concentration; $t_{\text{inset}}$, time of first detection; $t_{\text{last detection}}$, time of last detection.

Received July 5, 2011; accepted September 13, 2011. Previously published online at DOI: 10.1373/clinchem.2011.172254
MDMA metabolism influences MDMA-induced neurotoxicity (10, 11). Therefore, MDMA metabolism may be an important determinant in its neurotoxicity (12–15).

In vivo and in vitro studies with MDMA have revealed 2 main metabolic pathways (16). One major pathway includes multiple cytochrome P450–enzyme catalyzed O-demethylation to 3,4-dihydroxymethamphetamine (DHMA) followed by catechol-O-methyltransferase–catalyzed O-methylation mainly to 4-hydroxy-3-methoxymethamphetamine (HMAA). DHMA and HMAA can be further conjugated by uridine diphosphate glucuronoyltransferases (UGTs) to DHMA 3-glucuronide, DHMA 4-glucuronide, and HMAA glucuronide or by sulfotransferases to DHMA 3-sulfate, DHMA 4-sulfate, and HMAA sulfate. A minor pathway includes demethylation to 3,4-methylenedioxyamphetamine (MDA) followed by demethylation to 3,4-dihydroxyamphetamine (DHA), O-methylation to 4-hydroxy-3-methoxymethamphetamine (HMAA), and conjugation (5, 17–21).

To date, several pharmacokinetic studies in blood and urine after controlled MDMA administration to humans have been performed, mainly focused on MDMA and MDA (1) or additionally HMAA and HMAA (22–24). Few data are available for urinary excretion of DHMAA (25). In all studies, DHMAA, HMAA, and/or HMAA urinary pharmacokinetic data were obtained after conjugate cleavage either by acid or enzymatic hydrolysis with Helix pomatia. Only Shima et al. (19) determined HMAA’s intact glucuronides and sulfates in 25 random urine samples sent for forensic analysis, finding 72.7%–99.3% of HMAA excreted as conjugates. Therefore, we aimed to characterize urinary pharmacokinetics of MDMA and unconjugated phase I metabolites and phase II metabolites as intact glucuronides and sulfates after controlled oral MDMA administration to humans.

Materials and Methods

CHEMICALS AND REAGENTS

We obtained hydrochlorides of racemic MDA, HMAA, DHA, MDMA, HMAA, and DHMAA from Lipomed; methanolic solutions (1 g/L) of MDA-d₅ and MDMA-d₅ from LGC Promochem; and 4-hydroxymethamphetamine (pholedrine), 3,4-dihydroxybenzylamine, morphine 6-glucuronide, and hexamethyldisilazane from Sigma Aldrich. DHMAA sulfates, HMAA sulfate, and HMAA glucuronides were synthesized in the authors’ laboratory as described (20, 21). S-Heptfluorobutyrylpropyl chloride also was prepared as described (26). Isolute Confirm C18 cartridges (500 mg, 3 mL) were obtained from Biotage. All other chemicals (analytical grade) were from Merck.

CONTROLLED ORAL MDMA ADMINISTRATION

The study was conducted by the Chemistry and Drug Metabolism Section, National Institute on Drug Abuse (NIDA), Baltimore, MD. The study design, study population, drug administration, and sample collection have been described (23). Briefly, participants were administered placebo, 1.0 mg/kg (low), and 1.6 mg/kg (high) MDMA at least 1 week apart in this double-blind, randomized, placebo-controlled study. Urine samples from 10 participants were collected in institutional review board–approved protocols between 2005 and 2006 at NIDA. All urine voids were stored in individual refrigerators, measured for total volume, and divided into aliquots that were placed into multiple tubes and frozen at −20 °C once near the end of the day and each morning, Monday to Friday. Thus, most specimens were frozen within about 12 h (after refrigeration at the time of collection); on weekends, they were refrigerated until Monday morning for a maximum of 72 h. Separate aliquots of the same samples (756 samples) were shipped frozen (never thawed and refrozen) for blind analysis in 2010 by liquid chromatography/high-resolution mass spectrometry (LC-HRMS) and gas chromatography/negative-ion chemical ionization mass spectrometry (GC-NICI-MS) for this research.

CREATININE DETERMINATION

We determined urinary creatinine by the Jaffe reaction (Cobas Creatinine, Roche Diagnostics) on a Roche Hitachi 917 (Roche Diagnostics).

SAMPLE PREPARATION AND ANALYSIS

Urine sample preparation was performed as described (16). Briefly, we analyzed urine samples by 2 different approaches: LC-HRMS for glucuronides and sulfates and GC-NICI-MS for MDMA and its unconjugated phase I metabolites. Both methods were fully validated, including selectivity, recovery, matrix effects, process efficiency, bias and imprecision, stabilities, and limits of quantification and detection (16).

DATA CORRELATION

For comparison with previously published data (23), we performed linear regression with GraphPad Prism 5.00 (GraphPad Software).

PHARMACOKINETIC ANALYSIS

We measured concentration maxima (Cmax), with and without normalization to 100 mg/dL (9 mmol/L) creatinine (Cmax100), for MDMA, DHMAA, DHMAA 3-sulfate, DHMAA 4-sulfate, HMAA, HMAA sulfate, HMAA glucuronide, MDA, DHA, and HMA. We determined times of maximum concentration (tmax),
with and without normalization to 100 mg/dL creatinine \( (t_{\text{max100}}) \), for all analytes. The time interval after dosing for the first positive sample was designated as the time of first detection \( (t_{\text{onset}}) \); the interval after dosing for the last positive sample was designated as the time of last detection \( (t_{\text{last detection}}) \).

We calculated the percentage of dose excreted in urine for different time intervals (24 h each) and total dose excreted over 5–7 days, depending on the time the participants stayed on the residential unit, by summing the amounts (\( \mu\text{mol} \)) excreted in all individual urine samples for each study participant and dividing by the administered dose.

We calculated percentages of urine samples positive for each analyte by dividing the number of positive urine samples by the total number of samples collected. Statistical data comparison was performed with GraphPad Prism 5.00 using a nonparametric Wilcoxon matched pairs test (95% CI).

\section*{IN VITRO–IN VIVO CORRELATION}

We calculated ratios of HMMA sulfate to HMMA glucuronide and DHMA 3-sulfate to DHMA 4-sulfate at each time point and for total amounts excreted. We compared calculated ratios to those estimated from previous in vitro experiments with human liver cytosol and human liver (20, 21).

\section*{Results}

\subsection*{DATA CORRELATION}

When the 756 urine samples from 10 participants previously analyzed after acidic cleavage (23) were reanalyzed blind for this study to directly measure MDMA’s phase I and intact phase II metabolites, MDMA and MDA concentrations from both studies were compared, yielding correlation coefficients \( (R^2) \) of 0.82 and 0.75, respectively. For HMMA, results from the previous study were compared with the sum of unconjugated HMMA, HMMA glucuronide, and HMMA sulfate quantified separately in the present study. A correlation of \( R^2 = 0.87 \) between the 2 data sets was observed (Fig. 1).

\subsection*{PHARMACOKINETIC ANALYSIS}

MDMA was identified in 63% of urine samples, unconjugated DHMA in 32%, DHMA 3-glucuronide in 51%, DHMA 4-glucuronide in 40%, DHMA 3-sulfate in 69%, DHMA 4-sulfate in 59%, unconjugated HMMA in 38%, HMMA glucuronide in 84%, HMMA sulfate in 98%, MDA in 42%, unconjugated DHA in 3%, DHA glucuronides in 13%, DHA sulfates in 32%, unconjugated HMA in 23%, HMA glucuronide in 51%, and HMA sulfate in 50%. The small percentage of DHA-positive samples provided insufficient data for calculation of kinetic parameters. Median times (range) of first and last detection and \( t_{\text{max}} \) for all analytes detected and \( C_{\text{max}} \) values for MDMA, DHMA, DHMA sulfates, HMMA, HMMA glucuronide, HMMA sulfate, MDA, and HMA are shown in Table 1. \( C_{\text{max}} \) and \( t_{\text{max}} \) were additionally calculated after normalization to 100 mg/dL (9 mmol/L) creatinine. The highest \( C_{\text{max}} \) values after the low dose were HMMA sulfate, DHMA 3-sulfate, MDMA, and HMMA glucuronide, in decreasing order. After the high dose, MDMA had the highest \( C_{\text{max}} \). Except for MDMA, no significant \( C_{\text{max}} \) differences were observed after low and high doses. Large intersubject variability was noted. Normalization of concentration to urinary creatinine did not decrease variation in \( C_{\text{max}} \) (Table 1). Values of \( t_{\text{max}} \) without creatinine normalization ranged between 4 and 23 h for all analytes, whereas normalization resulted in a considerably earlier median \( t_{\text{max}} \) (<14 h) for all analytes. The effect of creatinine normalization on concentration–time profiles is exemplified in Fig. 2 for participant 3F after the 1.0- and 1.6-mg/kg doses.

Time of first detection (approximately 1–2 h) was comparable for MDMA and major metabolites. Only the lower-concentration metabolites were detected considerably later. HMMA sulfate had the longest detection time, up to 168 h. Except for HMA, sulfates could be detected longer than glucuronides.

\subsection*{PERCENTAGE TOTAL EXCRETION IN HUMAN URINE}

Urinary recovery of MDMA and metabolites over 5 days ranged between 24% and 52% of the MDMA dose (Fig. 3). Of the total MDMA recovered in urine, 46%
Table 1. Urine concentration $C_{\text{max}}$, $C_{\text{max}100}$, $t_{\text{max}}$, $t_{\text{max}100}$, $t_{\text{onset}}$, and $t_{\text{last detection}}$ of all metabolites detected in urine after low (1.0 mg/kg) and high (1.6 mg/kg) oral doses of MDMA.\(^a\)

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<tr>
<th>Dose</th>
<th>$C_{\text{max}}$ μmol/L</th>
<th>$C_{\text{max}100}$ μmol/L/100 mg/dL</th>
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<tr>
<td>MDMA</td>
<td>Low 56.9 (30.2–105.9) 83.2 (24.7–421) 12.6 (6.0–16.9) 7.4 (2.2–13.4) 1.4 (0.8–3.2) 98.8 (54.6–143.1)</td>
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<td>High 96.6 (36.1–283.1) 252 (106–427) 22.9 (3.1–30.4) 5.6 (0.1–9.1) 1.2 (0.6–5.0) 88.1 (56.8–167.1)</td>
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<td>DHMA</td>
<td>Low 1.1 (0.2–2.4) 1.5 (0.5–10.8) 13.7 (3.2–23.3) 13.7 (6.6–87.8) 1.6 (1.0–13.7) 47.3 (24.4–101.9)</td>
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<td>High 1.3 (0.5–3.2) 2.5 (0.7–8.7) 18.4 (3.1–30.4) 7.1 (1.3–35.2) 1.3 (0.6–6.8) 55.9 (33.3–86.6)</td>
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<td>DHMA 3-sulfate</td>
<td>Low 31.0 (18.8–66.0) 225 (27.9–381) 5.3 (1.5–16.6) 4.7 (2.2–10.0) 1.4 (0.4–3.2) 135.2 (71.0–106.6)</td>
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<td>High 43.4 (23.6–80.7) 210 (52.6–568) 5.0 (3.1–22.0) 5.1 (1.3–7.3) 1.2 (0.6–3.2) 101.3 (84.3–129.4)</td>
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<td>DHMA 4-sulfate</td>
<td>Low 6.7 (5.0–14.5) 28.8 (6.6–54.3) 13.2 (3.2–23.3) 7.9 (2.3–34.3) 1.4 (0.8–3.2) 72.4 (51.9–95.2)</td>
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<td>High 10.3 (4.8–19.7) 31.8 (5.9–52.3) 12.9 (3.1–30.4) 4.8 (1.3–7.3) 1.2 (0.6–3.2) 81.5 (55.9–105.1)</td>
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<td>DHMA 3-glucuronide</td>
<td>Low 11.5 (4.9–23.3) 6.6 (2.3–13.4) 4.7 (2.2–13.4) 2.0 (1.0–7.3) 1.4 (0.4–3.2) 57.2 (46.9–107.1)</td>
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<td>HMMA</td>
<td>Low 3.5 (1.1–20.5) 18.6 (0.6–49.5) 4.5 (1.5–9.2) 3.2 (1.5–7.3) 1.4 (0.8–3.2) 56.1 (30.0–95.0)</td>
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<td>High 8.2 (1.3–31.2) 41.6 (6.7–158.2) 3.9 (2.9–5.3) 4.4 (2.9–11.3) 1.2 (0.6–3.2) 66.1 (38.1–78.0)</td>
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<td>HMMA sulfate</td>
<td>Low 45.7 (28.6–84.6) 203.6 (46.0–445) 12.1 (2.1–23.3) 5.7 (2.2–17.3) 1.3 (0.4–3.2) 122.4 (83.6–167.6)</td>
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<td>High 66.7 (27.6–100.1) 187.0 (70.7–350) 9.5 (3.1–30.4) 5.3 (2.1–7.3) 1.2 (0.6–3.2) 150.1 (57.3–168.4)</td>
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<td>HMMA glucuronide</td>
<td>Low 32.8 (4.3–83.3) 82.0 (35.3–390) 7.3 (2.1–23.3) 4.7 (2.2–10.0) 1.4 (0.8–3.2) 102.7 (77.8–167.2)</td>
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<td>High 31.6 (5.0–60.0) 126.0 (28.5–295) 8.2 (3.1–30.4) 5.2 (3.1–7.3) 1.2 (0.6–3.2) 125.8 (81.3–168.4)</td>
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<td>MDA</td>
<td>Low 3.7 (2.0–11.8) 5.3 (1.9–30.6) 15.9 (10.6–25.5) 13.3 (6.0–34.3) 2.8 (1.0–8.3) 54.2 (32.2–65.2)</td>
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<td>HMA</td>
<td>Low 0.2 (0.1–0.2) 0.2 (0.1–1.4) 18.5 (5.4–39.1) 15.9 (5.1–94.3) 5.2 (1.5–24.6) 37.8 (16.5–129.5)</td>
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\(^a\) Data are median (range).  
\(^b\) Not determined.  
\(^c\) Data not sufficient for calculation of the kinetic parameters.
and 36% were excreted between 0–24 h after the low and high doses, respectively. Percentages decreased on subsequent days, 6% and 9% between 24 and 48 h, 0.7% and 1.2% between 48 and 72 h, 0.1% and 0.3% between 72 and 96 h, and <0.1% between 96 and 120 h. After 1.0 mg/kg MDMA, most of the dose was excreted in urine as HMMA sulfate (13%), followed by DHMA 3-sulfate (9%), MDA (8%), and HMMA glucuronide (5%). A greater percentage of the dose was excreted as unchanged MDMA after the high dose (11% vs 8%), whereas excretion of HMMA sulfate (10% vs 13%) and HMMA glucuronide (4% vs 5%) were lower.

Fig. 2. Concentration–time profiles of MDMA, MDA, DHMA, DHMA 3-sulfate, DHMA 4-sulfate, HMMA, HMMA sulfate, and HMMA glucuronide for participant 3F after the 1.0 mg/kg (A) and 1.6 mg/kg (B) MDMA dose, each without (upper) and with (lower) creatinine normalization.
after the high dose \((P = 0.02)\). No significant differences were observed for DHMA sulfate excretion between low and high doses. Unconjugated DHMA and HMMA accounted for <1% of the dose. In total, the median conjugation rate was 98% (range 96%–100%) for DHMA and 96% (range 88%–98%) for HMMA. No differences in conjugation rates were observed between the low and high doses.

**URINARY RATIOS**

The percentages of DHMA conjugates to total DHMA and HMMA conjugates to total HMMA were calculated at each time point and were consistent among the 10 participants. Conjugation rate did not differ between low and high doses and showed no changes over excretion time. In human urine, 96% (median, range 62%–99%) of DHMA was excreted as sulfates and 98% (57%–99%) of HMMA as glucuronide or sulfate.

Urinary ratios of DHMA 3-sulfate/DHMA 4-sulfate were calculated at each time point. The highest ratio was 26 (median 10) between 2 and 6 h after administration. Later, the ratio decreased to a median of 3. Expected ratios calculated from previous in vitro experiments in human liver cytosol \((20)\) were approximately 10. The median DHMA 3-sulfate/DHMA 4-sulfate urinary ratio, calculated from total excreted dose, was 4.5.

The HMMA sulfate/HMMA glucuronide ratios from in vitro studies (Fig. 4A) ranged from 10 to 2 at higher substrate (HMMA) concentrations \((20, 21)\). Urinary HMMA sulfate/HMMA glucuronide ratios calculated at each time point are shown in Fig. 4B. The highest ratios up to 15 (median 10) were generally detected within the first 2 h of excretion, decreasing to median ratios of 2 with increasing excretion time. The actual in vitro–in vivo HMMA sulfate/HMMA glucuronide ratio correlation (Fig. 4C) showed a median urinary ratio of 2, calculated from total amounts excreted. Considerable intersubject variability was observed, however; 1 participant had considerably higher ratios as shown in Fig. 4, B and C.

**Discussion**

Several studies have been performed to determine urinary excretion kinetics following controlled MDMA
administration; in all, however, DHMA, HMMA, or HMA pharmacokinetic data were obtained after conjugate cleavage by either acid or enzymatic hydrolysis with Helix pomatia (22–25). Our study is the first to monitor MDMA’s phase I and phase II metabolites after controlled low- and high-dose MDMA administration to humans.

Direct analysis of intact conjugates was a prerequisite for assessing the impact of glucuronidation and sulfation of MDMA’s phase I metabolites. The different conjugates represent individual chemical entities with their own risk of toxicity or drug interactions. From an analytical point of view, direct analysis allowed shorter analysis time because of simpler sample workup. In addition, possible variations in the extent of conjugate cleavage could be overcome (27).

All urine samples of the previous study (23) were stored at −20 °C before analysis. A critical point might be the stability of glucuronides and sulfates under these conditions. The aliquots shipped for reanalysis were never thawed and refrozen. In addition, freeze–thaw stability experiments conducted during the method validation process showed stability over at least 3 freeze–thaw cycles (16, 28). Long-term stability experiments previously conducted showed no instability over 6 months (16). Stability studies were performed recently over 13 months, and again no degradation was observed. Furthermore, good correlation was observed between the first analysis within 3–30 months after sample collection (2005–2006) published in 2009 (23) and the current analysis, indicating stability of the free analytes over a longer time period. Concerning the phase II metabolites, instability should result in the formation of the respective free drugs. In this study, however, concentrations of free drugs were <5% of total DHMA and HMMA, which further indicates a sufficient stability of the phase II metabolites.

All metabolites of MDMA—namely DHMA, HMMA, DHA, and HMA—were detected as glucuronide and sulfate conjugates. Because reference calibrators of the phase II metabolites were not commercially available and synthesis of these had to be performed in the authors’ laboratory, quantification focused on the main phase II metabolites DHMA 3-sulfate, DHMA 4-sulfate, HMMA sulfate, and HMMA glucuronide. However, tmax and first and last detection times were determined for all metabolites (Table 1) after identification via accurate masses of protonated molecular ions and daughter ions. Statistical comparison was performed by use of a nonparametric Wilcoxon matched pairs test, because it is independent of data distribution.

Although the median Cmax of MDMA was significantly higher (P = 0.002) after high-dose MDMA, no significant differences in Cmax values were observed after low and high doses for DHMA 3-sulfate, DHMA 4-sulfate, HMMA sulfate, and HMMA glucuronide. Among the conjugates, the highest concentrations (Table 1) were observed for HMMA sulfate, followed by DHMA 3-sulfate and HMMA glucuronide. DHMA 3-sulfate was significantly higher (P = 0.002) than DHMA 4-sulfate. However, MDMA Cmax values exceeded those of HMMA sulfate after low and high doses. In the same samples analyzed previously, the median HMMA Cmax was slightly higher than the median MDMA Cmax after the low dose (23). This Cmax was the sum of HMMA and its conjugates determined after hydrolysis. Direct measurement and summation of HMMA, HMMA sulfate, and HMMA glucuronide measured gave the same results as previously determined (Fig. 1). Urinary HMMA sulfate and glucuronide concentrations have been published (19) in 25 random human urine samples with unknown MDMA dose and ingestion time. HMMA sulfate and glucuronide concentrations ranged from 7 to 100 μmol/L and 1 to 40 μmol/L, respectively, and were comparable with our data. Only 1 of the 25 random urine samples had concentrations much higher than 200 μmol/L HMMA sulfate and 90 μmol/L HMMA glucuronide. No data are available for DHMA glucuronide and sulfate concentrations. Segura et al. (25) concluded from different cleavage procedures that DHMA glucuronides were minor metabolites. In our study, both DHMA 3-glucuronide and DHMA 4-glucuronide were detected following controlled MDMA administration. Assuming similar ionization properties between DHMA sulfate and DHMA glucuronide, DHMA glucuronides were <10% of DHMA sulfate concentrations.

Large intersubject variability was observed for all analyte urinary concentrations. Normalization of concentrations to urinary creatinine did not decrease this variation (Table 1). However, creatinine normalization generally decreased tmax variation and yielded considerably earlier median tmax values within the first 12 h of excretion (Table 1). In addition, more consistent concentration–time profiles were observed, as exemplified in Fig. 2B. Creatinine normalization had no major effect for only 2 participants after the low dose (Fig. 2A).

The major proportion of total urinary recovery occurred in the first 24 h. To obtain comparable data for all participants, total urinary recovery was calculated over 5 days. Urine samples were collected over 7 days for 7 participants, yet <0.1% of total MDMA dose was excreted after day 5. After the low dose, most MDMA was excreted in urine as HMMA sulfate (13%), whereas after the high dose, a greater percentage was excreted unchanged and the percentages of HMMA sulfate and glucuronide were significantly lower (P = 0.002). The higher percentage of unchanged MDMA is best explained by MDMA’s nonlinear kinetics (23, 29, 30).
The significantly lower HMMA sulfate and glucuronide percentages, combined with no significant differences in DHMA and DHMA sulfate percentages, might indicate inhibition of catecol-O-methyltransferase by DHMA, also demonstrated in vitro (31). Unconjugated DHMA and HMMA accounted for <1% of the dose, yielding 96% to 100% for DHMA conjugates and 88% to 98% for HMMA conjugates. Urinary ratios of HMMA/DHMA sulfates, DHMA 3-sulfate/DHMA 4-sulfate, and HMMA sulfate/HMMA glucuronide were calculated at each time when both analytes were quantifiable. For HMMA/DHMA sulfates, ratios were approximately 1.4 (median). DHMA 3-sulfate/DHMA 4-sulfate ratios revealed selective sulfation in position 3, in line with previous in vitro observations (20). Because the major proportion of total urinary recovery occurred in the first 24 h, in vitro–in vivo correlation was focused on this excretion period. For DHMA 3-sulfate/DHMA 4-sulfate ratios calculated from in vitro metabolism, data generally were similar to the actual in vivo ratios, although the in vivo data tended to be slightly lower (median 5.3) than the expected ratio of 10.

Median HMMA sulfate/HMMA glucuronide ratios of 10 (range 4–14) were observed shortly after MDMA administration, decreasing to 2 after approximately 4 h and staying constant thereafter (Fig. 4B). Shima et al. (19) recently published mean HMMA sulfate/HMMA glucuronide ratios in 25 random urine samples of 3.1 (1.8), in line with values determined in our study. In the previous in vitro experiments, the contribution of glucuronidation and sulfation were calculated for a range of expected HMMA plasma concentrations of 1–10 μmol/L. The respective HMMA sulfate/HMMA glucuronide ratios were 10 at lower HMMA concentrations, decreasing to a ratio of 2 with increasing HMMA concentration (20, 21) (Fig. 4A). This is in agreement with the observed urinary in vivo data. Referencing HMMA time–concentration curves following controlled MDMA administration, increasing HMMA concentrations were observed within 0–3 h (7, 29), explaining the higher urinary HMMA sulfate/HMMA glucuronide ratio within the first hours of excretion. Only 1 of 10 participants showed considerable variances, with significantly higher HMMA sulfate/HMMA glucuronide ratios (P < 0.001), whereas HMMA sulfate Cmax was comparable to those of other participants (35 μmol/L compared to median 55 μmol/L). However, HMMA glucuronidation Cmax in this individual was only 4 μmol/L (median 32 μmol/L). HMMA glucuronidation was primarily catalyzed by UGT2B15 (21), known to be polymorphically expressed (32). The phenotypic outcome of this polymorphism is still controversial; however, this might be an explanation for the different HMMA glucuronidation capacities observed for this 1 participant. Actual in vitro–in vivo correlation within the first 24 h of in vivo excretion is shown in Fig. 4C, indicating that ratios calculated from in vitro data generally were in the same range as those observed in vivo. Nevertheless, in vivo data tend to be lower (median ratio 2). Eliminating the 1 participant with HMMA sulfate/HMMA glucuronide ratios of about 8 had no relevant effect on the median HMMA sulfate/HMMA glucuronide ratio, which decreased to 1.7. However, it should be noted that, in the in vitro experiments, only initial metabolite formation could be monitored, and further pharmacokinetic processes such as distribution and excretion were neglected. In the in vivo experiments, only the sum of metabolism and distribution processes was determined.

In summary, ours are the first data obtained from evaluating phase II metabolism of MDMA in human MDMA users. Human MDMA urinary metabolites are primarily sulfate and glucuronide conjugates, with sulfates present in higher concentrations than glucuronides. Correlation with previous in vitro data by human liver microsomes and human liver cytosol showed that the ratios of the phase II metabolites were in the same range. This new knowledge will lead to improved urine MDMA and metabolite test interpretation in clinical and forensic toxicology, particularly for the performance of direct urine analysis of MDMA metabolites.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: M.R. Meyer, HOMFOR 2010, the research fund of the Medical Faculty, Saarland University, provided chemicals and reagents used for the determination of MDMA and its metabolites; M.A. Huetsis, Intramural Research Program of the NIH, NIDA, funded the controlled oral MDMA administration study.
Expert Testimony: None declared.
Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Acknowledgments: The authors thank Julia Dinger, Dirk K. Wissenbach, Carsten Schröder, Armin Weber, and Gabriele Ulrich from the Saarland University and Frank T. Peters from Schiller University Jena for assistance and discussions; we thank Thermo Fisher for providing the LC-HRMS.
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