Dose-response pharmacological study of mephedrone and its metabolites: pharmacokinetics, serotonergic effects and impact of *CYP2D6* genetic variation

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Key words: Mephedrone, Dose-response, Pharmacology, CYP2D6

31   **Abstract**

32   Mephedrone, the most widely-consumed synthetic cathinone, has been associated with acute
33   toxicity episodes. The aim of this report was to study its metabolic disposition, and the impact
34   of genetic variation of *CYP2D6* on MEPH metabolism, in a dose range compatible with its
35   recreational use.

36   A randomized, crossover, phase I clinical trial was performed. Subjects received 50 and 100 mg
37   (n=3) and 150 and 200 mg (n=6) of mephedrone and were genetically and phenotypically
38   characterized for the *CYP2D6* allelic variation.

39   Our results showed a linear kinetics of mephedrone at the dose range assayed: plasma
40   concentrations, cardiovascular and subjective effects, and blood serotonin concentrations all
41   correlated in a dose-dependent manner. Mephedrone metabolic disposition is mediated by
42   *CYP2D6*.

43   Mephedrone pharmacology presented a linear dose-dependence within the range of doses tested.
44   The metabolism of mephedrone by *CYP2D6* implies that recreational users with no or low
45   *CYP2D6* functionality are exposed to unwanted acute toxicity episodes.

1. Introduction

In 2008, due to a shortage of ecstasy and cocaine, mephedrone (MEPH) appeared as a new psychoactive-emerging drug(1,2). M-cat, as it is commonly called, is one of the most widely-used drugs located within a family group of new psychoactive substances known as synthetic cathinones(3). Despite the fact that worldwide MEPH is either banned or controlled, its consumption has steadily risen and is now apparently stabilized. The number of associated deaths has, however, recently increased(4,5). It is the established drug of choice in several user populations (e.g. chemsex sessions(6)), and may be consumed via oral, intranasal, and intravenous routes, alone or in combination with other psychoactive drugs(4).

MEPH produces physiological (e.g. increased arterial blood pressure, heart rate, pupil diameter, and body temperature) and subjective effects (e.g. stimulant-like results) typical of a psychostimulant drug although with a faster onset and shorter duration when compared to MDMA(7,8). The metabolic disposition of MEPH in animal models is characterized by low bioavailability via the oral route, and an extensive hepatic metabolism (10). Its metabolism has been described in rats(9,10), liver hepatocytes(11), human liver microsomes(12), and human biological samples(13). MEPH is metabolized in the liver and the major metabolic reactions are N-demethylation, hydroxylation, oxidation, reduction, and conjugation with carboxylic acids and glucuronides (13,14) (see Figure 1). The cytochrome P450 2D6 (CYP2D6), a highly polymorphic drug metabolizing enzyme(15), has been described *in vitro* as the main enzyme responsible for the phase I metabolism of MEPH with some other contributions from NADPH-dependent enzymes(12). CYP2D6 has been also described to be implicated in the metabolism of other synthetic cathinones (such as methylone)(16). The role of *CYP2D6* polymorphism regarding MEPH disposition in humans, and its potential clinical relevance, has not yet been evaluated.

Recently, our research group described the kinetics in humans of MEPH and several metabolites [nor-mephedrone (NOR-MEPH), dihydro-mephedrone (DIHYDRO-MEPH), 4-carboxy-

mephedrone (COOH-MEPH), and succinyl-nor-mephedrone (SUCC-NOR-MEPH)] both in plasma and urine after the ingestion of 150 mg of MEPH(17). It is currently unknown, however, whether the recovery of metabolites varies in a dose-dependent manner.

Whilst MEPH pharmacokinetics has been investigated in animal models, and following a bi-compartmental model(10), research regarding different dosages in humans is pending. Reported MEPH oral doses vary greatly from 15 to 250 mg (5,18) and it is unknown whether, in a similar manner to MDMA, MEPH pharmacokinetics is non-linear(19).

We report a dose-dependent pharmacology study of MEPH and its metabolites in healthy subjects administered with different doses of the drug (50 mg and 100 mg n=3; 150 mg and 200 mg n=6). Data regarding MEPH and metabolite concentrations in plasma and urine have been used to describe its pharmacokinetics. Furthermore, pharmacodynamics parameters (cardiovascular and subjective effects) and 5-HT concentrations in human blood, were monitored after the administration of MEPH doses and correlated with MEPH and metabolite concentrations and the subjects' CYP2D6 phenotype.

2. Results

2.1. Pharmacokinetics of MEPH and its metabolites

The time course of plasma concentrations of MEPH, NOR-MEPH, SUCC-NOR-MEPH, DIHYDRO-MEPH, and COOH-MEPH are presented in Figure 2 after single doses of 50, 100, 150, and 200 mg of MEPH. Experimental pharmacokinetic parameters [Peak concentrations (C_{max}), time to reach peak concentrations (T_{max}), and area under the curve (AUC_{0-8h})] of MEPH and metabolites are described for all volunteers in Table 1.

With respect to MEPH kinetics, peak concentrations were reached around 1h post drug administration. Peak plasma concentrations and the amount of drug recovered in urine, increased with the doses administered. Regarding MEPH metabolites, COOH-MEPH was the most abundant. Plasma concentrations of COOH-MEPH appeared rapidly (T_{max} around 1h at all doses). Concerning DIHYDRO-MEPH and NOR-MEPH, both compounds displayed similar

pharmacokinetic behaviors, the latter being 5 times more abundant than the former. The experimental pharmacokinetics of SUCC-NOR-MEPH indicated a residual formation of this metabolite (with the lowest $C_{\max}$ and AUC_{0-8h} values) and later peaking plasma concentrations ($T_{\max}$ of around 4h post administration).

In urine, MEPH and metabolites recovery was proportional to the doses administered (see Figure 3).

2.2. Pharmacokinetics of MEPH

MEPH plasma concentrations followed a bicompartamental model. With respect to the calculated pharmacokinetic parameters, MEPH presented a similar elimination constant rate (K_e) (at $0.3h^{-1}$ approx.) and elimination half-life ($t_{1/2}$) (of 2h approx.) irrespective of the administered dose (see Table S2). However, both plasmatic clearance ($Cl_{\text{plasmatic}}$) and volume of distribution (V_d) varied notably among individuals. Considerable dispersion was observed among those who received the lowest doses ($n=3$) which could have been due to the reduced sample size ($n=3$). It might also have been explained by the different MEPH concentrations in plasma among the volunteers' AUC_{0-8h} which went from 16 ng·h/mL (for id 3, *CYP2D6* ultrarapid metabolizer) to 222 ng·h/mL (id 1, *CYP2D6* normal metabolizer). In fact, the exclusion of the ultrarapid metabolizers resulted in similar $Cl_{\text{plasmatic}}$ (mean 31.2 L/h compared to that observed for 150 and 200 mg) irrespective of the administered dose suggesting a linear pharmacokinetic behavior of MEPH in the assayed dose range. The urinary excretion of unaltered MEPH ranged from 5 to 15% among doses.

2.3. Pharmacodynamics of MEPH

The statistical analysis of the effects elicited by the four tested doses is reported in Table S3. With respect to the physiological effects, the administration of all doses (50, 100, 150 and 200 mg of MEPH) produced significant changes in subjective and cardiovascular parameters. Figure 4 depicts the maximum effect ($E_{\max}$) of cardiovascular parameters such as systolic blood pressure (SBP) and heart rate (HR), and of 'high' subjective effects, and their correlation with

the MEPH $C_{\max}$ within the tested dose range. The monitored cardiovascular parameters correlated with the dose increment of MEPH [Spearman correlation coefficient, $Rho=0.563$, $p < 0.001$ for SBP and $Rho=0.879$, $p < 0.001$ for HR]. Furthermore, subjective effects also positively correlated with the plasma concentrations of MEPH [$Rho=0.879$, $p < 0.001$ for VAS 'high', $Rho=0.783$, $p < 0.001$ for VAS 'good effects', and $Rho=0.805$, $p < 0.001$ for VAS 'stimulation']. Results are shown in Table S3.

Concerning free 5-HT in blood, the administration of 50 and 100 mg of MEPH did not significantly change basal values. However, doses of 150 and 200 mg of MEPH raised 5-HT blood concentrations in a dose-dependent manner. Such an increment was dose-dependent with a 3 to 5-fold $E_{\max}$ increase in 5-HT concentrations above baseline (14.5 ± 7.6 nM and 23.5 ± 7.8 nM, respectively). Both MEPH and 5-HT $C_{\max}$ and $E_{\max}$ peaked at 1h post-drug administration. Moreover, a significantly positive correlation between 5-HT blood and MEPH plasma concentrations was observed ($Rho=0.811$, $p=0.01$).

Regarding the MEPH metabolites we found no correlation between any metabolite concentrations and the variations in physiological and subjective effects.

2.4. Impact of *CYP2D6* phenotype on MEPH pharmacokinetics

The dose-normalized AUC_{0-8h} was higher in subjects with two normal function alleles (i.e. those with an activity score of 2 vs. subjects carrying at least one decreased or no function allele (i.e. those with an activity score of 1 or 1.5), suggesting that MEPH disposition is mediated by *CYP2D6*. This was observed both in the population of 9 subjects ($p=0.001$) of this report [where five volunteers had genotypes predicting normal or ultrarapid metabolism ($AS \geq 2$ and four subjects had genotypes predicting various degrees of decreased activity (AS of 1 or 1.5)] and in a larger population of 20 subjects [where $n=10$ had an AS of ≥ 2 and $n=10$ had an AS of 1 or 1.5] which included additional participants from other MEPH clinical studies(7). The normal metabolizers ($AS \geq 2$) were those displaying the lowest AUC_{0-8h} values (Figure 5a). Furthermore, with respect to the subjective effects (VAS High), identical results were obtained

for the different *CYP2D6* metabolizers groups (Figure 5b). Overall, subjects with lower CYP2D6 activity score had higher MEPH plasma levels and greater augmented effects compared to those having 2 functional gene copies(20,21).

The ratios of AUC_{0-8h} of metabolites vs. MEPH (100 mg for n=3 subjects and 200 mg for n=6 subjects, only these doses were chosen in order to avoid duplicate subjects) showed that they were significantly lower for NOR-MEPH (p = 0.021), SUCC-NOR-MEPH (p<0.001) and COOH-MEPH (p<0.001) but not for DIHYDRO-MEPH in individuals with an AS of ≤ 1.5 (Figure 6).

3. Discussion

We tested four doses of MEPH in humans in a pilot dose-finding study prior to a larger study designed to evaluate MEPH clinical pharmacology compared to MDMA(7). We report for the first time that physiological and subjective effects, and serotonin blood concentrations, in humans correlate in a dose-dependent fashion with MEPH plasma concentrations. We also describe for the first time that MEPH metabolic disposition in humans is impacted by *CYP2D6* allelic variation.

With respect to pharmacodynamic effects, we found a proportional increase between MEPH concentrations (C_{max}) and the effects (E_{max}) elicited by the drug. That is to say, a positive correlation between drug concentrations in plasma at each tested MEPH dose and results for subjective (VAS high, good effects, stimulated) and cardiovascular (SBP and HR) effects was observed (Figure 4). We did not, however, observe a correlation between MEPH metabolites, including NOR-MEPH which is reportedly pharmacologically active (25) and the monitored pharmacodynamic effects.

MEPH, like MDMA, induces a rapid release and elimination of serotonin in rat brain(22). In humans we have already described that MDMA leads to an increase in the gene expression of the serotonin transporter in blood platelets(23). In our present work, we observed a dose-

dependent release of serotonin from platelets after MEPH administration. Concentrations of 5-HT peaked at 1h post MEPH administration, similarly to the 5-HT increase after MDMA administration.

The pharmacokinetics of MEPH and its metabolites was investigated after the administration of four different doses (50, 100, 150 and 200 mg). MEPH was rapidly absorbed at all doses, plasma concentrations peaked at around 1h post-drug administration, and the elimination process was rapid, in accordance with previous studies(7,17,24). The profile of metabolites recovered in urine was similar for all doses tested. The poor and variable bioavailability of MEPH by the oral route in rats(10), about 6 times lower than for MDMA(10,24), and its normal metabolism(13) partially mediated by CYP2D6(12) and is likely contributing to a high inter-individual variability in its recovery. Despite the marked differences in the pharmacokinetics of MEPH, results suggest that its kinetics is linear within the range of doses tested (50-200 mg) in contrast to MDMA(19). MEPH exhibited non-linear pharmacokinetic behavior in rats at higher doses(10) that were unlikely to be ingested in humans unless in cases of acute intoxication.

In vitro studies suggest that the metabolic disposition of most amphetamine analogs and other psychostimulant drugs is metabolized by human liver CYP2D6(25). In agreement, CYP2D6 has been described *in vitro* as playing a key role in MEPH metabolism(12). In our study, we found that CYP2D6 activity significantly altered MEPH plasma concentrations ($AUC_{0-8h}/dose$) and its pharmacodynamic effect (Figure 5). Such a finding highlights the relevance of the individual *CYP2D6* genotype/phenotype: poor-metabolizers are expected to have higher concentrations and stronger MEPH effects which increase the risk of clinical complications associated with consumption and ultrarapid metabolizers are expected to re-dose more frequently in order to experience the desired effects. Moreover, blood concentrations of MEPH as for 3-methylmethcathinone (3-MMC) in cases of fatal intoxications were higher than in non-fatal cases(5,26), which could be caused due to the impact of genetic variation of CYP2D6. The calculated metabolite/parent compound ratios confirmed the implication of CYP2D6 in the two initial metabolic steps leading to the formation of NOR-MEPH and hydroxytolyl-MEPH (Figure

1). Hydroxytolyl-MEPH could not be measured in biological fluids because of a lack of reference materials. Nevertheless, the fact that the COOH-MEPH: MEPH ratio is modulated by CYP2D6 (Figure 6) is most likely due to the availability of its precursor hydroxytolyl-MEPH. The same would be the case for the SUCC-NOR-MEPH: MEPH ratio. The contribution of other enzymes to the metabolite formation cannot be ruled out. It appears, however, that both main pathway are mainly mediated by CYP2D6; of particular importance is the one leading to hydroxytolyl-MEPH and COOH-MEPH formation, the most relevant quantitatively in humans(16). Concerning DIHYDRO-MEPH, it appears that CYP2D6 was not involved in its formation as no alterations of the DIHYDRO-MEPH: MEPH ratio were found. MEPH accumulation in the body can, at least in part, be explained by *CYP2D6* genotype and thus, genetic variation appears to contribute to the large inter-individual variability in clinical toxicity associated with MEPH use(5,27). The considerable difference in MEPH bioavailability may obscure the effects attributable to CYP2D6 and its relevance in acute intoxication cases should be tuned down(28).

Our study has strengths and limitations. The main limitation is the number of participants, although findings regarding the impact of the genetic variation of CYP2D6 in the MEPH metabolism were confirmed in a second independent population. Taking into consideration that only subjects with decreased or normal functional CYP2D6 alleles were included for ethical and safety reasons, the results obtained are relevant regarding the clinical pharmacology of MEPH. The inclusion of poor metabolizers would have further strengthened our observations.

A larger sample size with women and the evaluation of other administration routes (such as insufflation) would provide valuable information. Moreover, the lack of analytical standards for other metabolites (such as hydroxytolyl-MEPH) made proper quantification unfeasible. Further studies with a larger population group would be required in order to study the pharmacological activity of the MEPH metabolites and their contribution to adverse effects. Nevertheless, our study has allowed the global evaluation of pharmacokinetic, pharmacodynamic, and

pharmacogenetic aspects of MEPH clinical pharmacology at a range of doses relevant among recreational users.

4. Materials and methods

4.1 Subjects and study design

Within the context of a pilot, dose-finding, MEPH study, nine healthy male subjects were recruited for a phase I, double-blind, clinical trial at the Clinical Research Unit of the Hospital del Mar Medical Research Institute (IMIM). Volunteers were given a single oral dose of MEPH per session and each session was separated at least by a week of wash-out (to minimize any carry-over effect). From the nine volunteers of the trial, three ingested 50 and 100 mg of MEPH and six 150 and 200 mg of MEPH in different sessions. Subjects were recreational users of amphetamines, ecstasy, MEPH, and other cathinones. Participants had an age range 34-41 years and weighed 63.6-73.5 kg. The present pilot study was followed by a clinical study where subjects ingested a dose of 200 mg of MEPH(7).

To be included in the study, volunteers were submitted to a general medical examination and a psychiatric interview (PRISM) to exclude any psychopathological condition. Inclusion and exclusion criteria have been previously described (7). The study protocol included urine drug testing: cocaine, cannabinoids, MDMA, and methamphetamine before participation in every experimental session. Subjects signed an informed consent prior to participation and were economically compensated for any inconvenience caused during the trial.

The study was approved by the local ethics committee (Clinical Research Ethical Committee - Parc de Salut Mar IMIMFTCL/MEF/1, 2013/5045) and registered in ClinicalTrials.gov (NCT02232789). The clinical trial was conducted in accordance with the Declaration of Helsinki and Spanish laws concerning clinical research.

Subjects were phenotyped with dextromethorphan and only those with a dextromethorphan/dextrorphan ratio >0.3 were included in the study. Poor metabolizers with a

urinary metabolic ratio of <0.3 (20,29) were excluded from the study for safety reasons. Additionally, CYP2D6 genotyping was performed using the PHARMachip DNA array (Progenika Biopharma S.A., Derio, Spain)(30). Following genotypes analysis, CYP2D6 activity scores were calculated for each subject and phenotypes were assigned to participating volunteers(31) and the activity score for CYP2D6 was calculated for each subject(21) (see Table S1). Due to the small number of subjects participating in this study, a second, independent set of 11 subjects was included (therefore the total population was 20 subjects) to confirm the genetic impact of *CYP2D6* on MEPH metabolic disposition. The first 9 subjects in Table S1 correspond to those of the dose-finding study we report, while the following subjects correspond to a larger study with MEPH 200mg(7).

4.2 Sample collection

In the experimental sessions, blood samples were collected at baseline (pre-administration) and 1, 2, 4, 6, and 8 h post MEPH administration; urine was also collected in the time periods: 0–4, 4–8, 8–12, 12–24, and 24–48 h post drug administration.

4.2. Drugs

MEPH was supplied by the Spanish Ministry of Justice and prepared by the Pharmacy Department (Hospital del Mar, Barcelona, Spain) as soft capsules.

4.3 Analytical methods

MEPH and metabolites were quantified in plasma and urine with a validated liquid chromatography tandem - mass spectrometry (LC-MS/MS) method(17). Free serotonin (5-HT) concentrations in blood human were quantified by high-performance liquid chromatography (HPLC) with electrochemical detection(32).

4.4 Pharmacokinetic modelling

The observed pharmacokinetic parameters, such as: $C_{\max}$, time to reach $T_{\max}$, and AUC_{0-8h} were obtained using Microsoft Excel functions(33).

Pharmacokinetic parameters of MEPH, NOR-MEPH, SUCC-NOR-MEPH, DIHYDRO-MEPH, and COOH-MEPH were calculated using a compartmental method with the aid of SAAMII (The Epsilon Group, <https://tegvirginia.com/software/saam-ii-popkinetics/>)(34). K_e was estimated by the sum of elimination constants of MEPH and the first order constants of metabolites. The $t_{1/2}$ was calculated as $0.693/K_e$. The area under the plasma AUC was calculated using the trapezoidal rule and was extrapolated to infinity. The apparent CL_p of MEPH was calculated as $F \cdot \text{Dose}/\text{AUC}$, and its metabolic ratio (MR) was calculated as the ratio of $\text{AUC}_{\text{metabolite}}$ to AUC_{MEPH} for each metabolite. $MEPH$ and Cl_r were calculated as the ratio of the cumulative excretion and the AUC from 0 to infinity. A bioavailability (F) of 10% was considered(10). In Figure S1, the pharmacokinetic profile of MEPH (observed vs modelled) is presented.

4.5. Pharmacodynamics

Blood pressure and heart rate were evaluated along the experimental sessions at baseline time (pre-dose) and at 1, 2, 4, 6, and 8 h post MEPH administration. All assessments were obtained using a Dinamap^{TM} 8100-T vital signs monitor (Critikon, Tampa, FL, USA). Subjective effects were evaluated using twenty-one 100-mm visual analogue scales (VAS) labelled with different adjectives (such as stimulant, high, or good effects). Subjects were asked to score effects they were experiencing at different time points (basal, 1, 2, 4, 6 and 8h) after drug administration(7).

4.6. Statistical Analysis

All data were analyzed using the SPSS Statistics 18.0 package (SPSS, Chicago, USA). The normality of variables was evaluated and non-parametric test applied. The Spearman correlation and the Kruskal-Wallis test was employed for pharmacodynamic analysis and the U-Mann Whitney test for the pharmacogenetics. A value of $p < 0.05$ was considered statistically significant.

5. Study highlights

- What is the current knowledge on the topic?

MEPH has a human pharmacology similar to MDMA, but with an earlier onset of effects that also vanish quickly.

- What question did this study address?

Are MEPH effects dose-dependent? Is its metabolic disposition mediated by CYP2D6?

- What this study adds to our knowledge?

There is a linear dose-dependent relationship between MEPH concentrations and effects. Plasma concentrations of MEPH strongly correlate with cardiovascular and subjective effects, and serotonin release from platelets. CYP2D6 regulates the main metabolic pathways of MEPH metabolic clearance.

- How this might change clinical pharmacology or translational science?

Because of the high correlation between plasma concentrations and physiological effects, subjects with a low CYP2D6 functionality are at risk of unwanted toxicological effects.

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Disclosure of potential conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and the 1964 Helsinki Declaration.

324   **Informed consent**

325   Informed consent was obtained from all individual participants in the study.

326   **Author contributions**

327   R.dT. and E.O. wrote the manuscript; M.F., M.T. and R.dT. designed the research; M.F, M.T,

328   E.P, C.P-M performed the research; OJ.P, S.Y-L, M.P, M.C and E.O analyzed the data; OJ.P,

329   R.dT. and E.O. contributed new analytical tools.

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454

Figure 1. Partial metabolic pathways of MEPH. MEPH, NOR-MEPH, SUCC-NOR-MEPH, DIHYDRO-MEPH and COOH-MEPH have already been identified and quantified both in plasma and urine.

Figure 2. Mean and SEM plasma concentrations of MEPH and metabolites (ng/mL) over time (h). All compounds were quantified at 0, 1, 2, 4, 6 and 8h after drug administration at different doses: 50, 100, 150 and 200 mg. Three subjects ingested 50 and 100 mg of MEPH, and six subjects ingested 150 and 200 mg.

Figure 3. Mean recovery (and SEM) of MEPH and its metabolites (in μ moles) in urine after the ingestion of different doses of MEPH (50, 100, 150 and 200 mg) at 0-8h post drug administration. Three subjects ingested 50 and 100 mg of MEPH, and six subjects ingested 150 and 200 mg.

469

470 **Figure 4.** Peak plasma concentrations for MEPH (grey circles) on the right Y axis and different
 471 pharmacodynamic parameters (at the time of C_{max}) (black squares) on the left Y axis, in relation
 472 to the dose administered in 9 subjects (3 for doses 50 and 100 mg and 6 for 150 and 200 mg).
 473 The represented physiological and subjective parameters were systolic blood pressure (SBP) in
 474 mmHg, heart rate (HR) in bpm, serotonin concentrations in blood ([5-HT]) in nM, and values of
 475 the visual analog scale (VAS high) in mm at E_{max}.

476

477 **Figure 5.** Scatter dot-spot plot of the plasma concentrations over time (AUC_{0-8h}) of MEPH and
 478 VAS 'high' (normalized by dose) following CYP2D6 phenotype (see Table S1 for details): a)
 479 Individual data of MEPH AUC_{0-8h} for subjects with an activity score of > 2 or 2 and those with
 480 an activity score of ≤ 1.5 (in grey for activity score = 1.5 and in black for an activity score = 1)
 481 carrying decreased and/or no function alleles. The circles correspond to the subjects from the
 482 pilot study (n=9) and in squares the additional MEPH study (n=11). b) Subjective effects (VAS
 483 'High') AUC_{0-8h} for fully CYP2D6 metabolizers (activity score ≥ 2) and for partial metabolizers
 484 (activity score ≤ 1.5).

485

486 **Figure 6.** Blog-spot of the metabolic ratios of MEPH metabolites according to CYP2D6 activity
 487 score (n= 4 volunteers with an activity score ≤ 1.5 and n=5 subjects with an activity score of ≥ 2).
 488 The ratios were calculated with the targeted metabolites (NOR-MEPH, SUCC-NOR-MEPH,
 489 COOH-MEPH and DIHYDRO-MEPH) with the parent compound (MEPH). Significant values
 490 obtained with a U-Mann Whitney are expressed by * when p value was ≤ 0.05 and by ** when
 491 the p value was ≤ 0.01 .

Compound	Parameters	Units	Dose			
			50 mg	100 mg	150 mg	200 mg
MEPH	C _{max}	ng/mL	37.4 ± 16.4	51.7 ± 20.5	179.0 ± 29.3	255.6 ± 70.0
	T _{max}	h	2 (1-2)	1 (1-2)	1 (1-2)	1 (1-2)
	AUC _{0-8h}	ng*h/mL	122.5 ± 59.7	169.4 ± 93.5	588.2 ± 93.4	879.4 ± 194.1
NOR-MEPH	C _{max}	ng/mL	20.6 ± 7.6	27.8 ± 12.8	54.4 ± 4.9	87.7 ± 23.8
	T _{max}	h	2 (1-2)	1 (1-2)	1.5 (1-2)	2 (1-2)
	AUC _{0-8h}	ng*h/mL	100.6 ± 45.5	132.8 ± 76.6	267.6 ± 32.3	380.7 ± 71.0
SUCC-NOR-MEPH	C _{max}	ng/mL	2.9 ± 1.3	3.3 ± 2.0	4.5 ± 0.5	8.0 ± 2.3
	T _{max}	h	4 (2-6)	2 (2-6)	4 (2-4)	4 (2-6)
	AUC _{0-8h}	ng*h/mL	15.6 ± 7.1	17.7 ± 10.4	23.8 ± 4.3	37.1 ± 7.7
DIHYDRO-MEPH	C _{max}	ng/mL	2.9 ± 1.2	4.2 ± 1.6	10.9 ± 1.6	19.7 ± 5.0
	T _{max}	h	2 (1-2)	1 (1-4)	1 (1-4)	1.5 (1-4)
	AUC _{0-8h}	ng*h/mL	15.8 ± 7.8	21.0 ± 11.5	61.7 ± 11.9	95.3 ± 24.9
COOH-MEPH	C _{max}	ng/mL	141.1 ± 59.8	168.2 ± 42.8	294.4 ± 30.2	292.6 ± 51.0
	T _{max}	h	2 (1-2)	1 (1)	1 (1-2)	1 (1-2)
	AUC _{0-8h}	ng*h/mL	283.3 ± 45.5	428.6 ± 115.2	662.1 ± 69.6	912.2 ± 128.0

492

493 **Table 1.** Experimental pharmacokinetic parameters of MEPH, NOR-MEPH, SUCC-NOR-
494 MEPH, DIHYDRO-MEPH and COOH-MEPH after a single oral administration of MEPH at
495 different doses (50, 100 (n=3) and 150, 200 mg (n=6)). Mean values ± SEM of C_{max} and AUC_{0-8h}
496 and median values and range of T_{max}.

Figure S1. Example of the pharmacokinetic profile of mephedrone and its metabolites (nor-mephedrone, N-succinyl-nor-mephedrone, dihydro-mephedrone and 4'-carboxy-mephedrone) in plasma and accumulated urine in one volunteer (id 6). The dashed line shows the fitted parameters and the full figures (circle, square, triangle and diamond) show the observed concentrations.

Subjects	Dose (mg)	CYP2D6 allele	Phenotype	Activity Score	DM/DX ratio
id 1	50/100	*1/*1	Normal	2	0.011
id 2	50/100	*1/*2	Normal	2	0.001
id 3	50/100	*1XN/*2	Ultrarapid	>2	0.002
id 4	150/200	*1/*9	Normal	1.5	0.009
id 5	150/200	*1/*4XN	Intermediate	1	0.004
id 6	150/200	*1/*2	Normal	2	0.002
id 7	150/200	*1/*41	Normal	1.5	0.005
id 8	150/200	*2/*4	Intermediate	1	0.008
id 9	150/200	*2/*2	Normal	2	0.011
id 10	200	*1XN/*2	Ultrarapid	>2	0.004
id 11	200	*1/*5	Intermediate	1	0.009
id 12	200	*1/*1	Normal	2	0.002
id 13	200	*2/*10	Normal	1.5	0.002
id 14	200	*1/*41	Normal	1.5	0.002
id 15	200	*1/*1	Normal	2	0.003
id 16	200	*1/*3	Intermediate	1	0.004
id 17	200	*2/*4	Intermediate	1	0.002
id 18	200	*2XN/*4	Normal	2	0.003
id 19	200	*1/*1	Normal	2	0.008
id 20	200	*1/*4	Intermediate	1	0.011

503

504 **Table S1.** Doses administered per subject and the corresponding CYP2D6 genotype/assigned
505 phenotype, activity score and dextromethorphan/dextrorphan (DM/DX) ratio.

MEPH' Pharmacokinetics Parameters	Units	Dose			
		50 mg	100 mg	150 mg	200 mg
K_e	h^{-1}	0.34 ± 0.02	0.36 ± 0.04	0.32 ± 0.01	0.35 ± 0.04
$t_{1/2}$	h	2.03 ± 0.12	1.99 ± 0.23	2.18 ± 0.07	2.12 ± 0.27
$Cl_{plasmatic}$	L/h	125.6 ± 95.1	97.2 ± 34.6	33.0 ± 10.2	33.0 ± 10.6
V_d	L	353.0 ± 263.8	256.1 ± 78.9	101.23 ± 28.17	98.1 ± 26.9
% Excretion	%	4.5 ± 3.5	7.5 ± 6.7	13.4 ± 4.6	14.0 ± 7.5

506

507 **Table S2.** Mean $\pm$ SEM values of the modelled pharmacokinetic parameters: K_e , $t_{1/2}$, $Cl_{plasmatic}$,
508 V_d , % Excretion (n=3 for 50 and 100 mg and n=6 for 150 and 200 mg). All volunteers that
509 ingested orally 50, 100, 150 and 200 mg of MEPH considering a hypothetical bioavailability of
510 10%.

Pharmacodynamic Parameters	E _{max} units	Dose				P values
		50 mg	100 mg	150 mg	200 mg	
SBP	mmHg	6.3 ± 7.4	9.7 ± 7.6	24.2 ± 2.4	31.6 ± 2.6	0.023
DBP	mmHg	4.7 ± 4.8	3.7 ± 5.1	10.2 ± 1.1	10.8 ± 1.56	0.558
HR	bpm	6.0 ± 3.1	10.0 ± 3.5	21.8 ± 6.4	27.8 ± 11.7	0.067
[5-HT]	nM	1.8 ± 0.6	0.3 ± 0.0	14.5 ± 7.6	23.5 ± 7.8	0.034
VAS 'high'	mm	1.3 ± 0.7	0.7 ± 0.0	13.0 ± 7.6	24.0 ± 10.8	0.013
VAS 'good effects'	mm	1.0 ± 1.0	0.0 ± 0.0	18.4 ± 12.8	26.2 ± 11.2	0.028
VAS 'stimulated'	mm	2.3 ± 2.3	0.0 ± 0.0	11.2 ± 7.2	20.6 ± 10.3	0.062

511 **Table S3.** Mean ± SEM values of the E_{max} values for cardiovascular and subjective effect
 512 parameters and 5-HT concentrations after the administration of different doses of MEPH (50,
 513 100, 150 and 200 mg). P values were calculated by a Kruskal-Wallis test.