REVIEW ARTICLE

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Clinical Pharmacokinetics of Amfetamine and Related Substances

Monitoring in Conventional and Non-Conventional Matrices

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Contents

Abstract

Consumption of amfetamine-type stimulants, including classical amfetamines and 'designer drugs', has been recognised as one of the most significant trends in drug abuse at the end of the past century and at the beginning of the current one. The first cause is the increasing consumption amongst youth of methylenedioxy- and methoxy-substituted amfetamines, of which the pharmacology in humans is currently under investigation. Secondly, the abuse of more classical amfetamines, such as amfetamine itself and metamfetamine, continues to be highly prevalent in some geographical regions.

Amfetamines are powerful psychostimulants, producing increased alertness, wakefulness, insomnia, energy and self-confidence in association with decreased fatigue and appetite as well as enhanced mood, well-being and euphoria. From a clinical pharmacokinetic perspective, amfetamine-type stimulants are rather homogeneous. Their oral bioavailability is good, with a high distribution volume (4 L/kg) and low binding to plasma proteins (less than 20%). The elimination half-life is 6–12 hours. Both hepatic and renal clearance contribute to their elimination from the body. Hepatic metabolism is extensive in most cases, but a significant percentage of the drug always remains unaltered.

Amfetamine and related compounds are weak bases, with a pKa around 9.9, and a relatively low molecular weight. These characteristics allow amfetamine-type stimulants to diffuse easily across cell membranes and lipid layers and to those tissues or biological substrates with a more acidic pH than blood, facilitating their detection in alternative matrices at relatively high concentrations. In most cases, the concentrations found are higher than expected from the Henderson-Hasselbach equation. Drug monitoring in non-conventional biological matrices (e.g. saliva, hair, nails, sweat) has recently gained much attention because of its possible applications in clinical and forensic toxicology. An individual's past history of medication, compliance or drug abuse can be obtained from testing of hair and nails, whereas data on current status of drug use can be provided by analysis of sweat and saliva.

Because of the physicochemical properties of amfetamine-type stimulants, this group of drugs is one of the most suitable for drug testing in non-conventional matrices.

Amfetamine (amphetamine; β-phenylisopropylamine) is the prototype of a class of compounds with central and peripheral stimulant activity.[1] These compounds, usually referred as amfetamines or amfetamine-type stimulants, include a number of structurally related substances (e.g. metamfetamine, fenfluramine, phentermine, synthetic amfetamine analogues and methylenedioxy derivatives) that have some pharmacological and toxic effects in common with amfetamine.

Ephedrine, a naturally occurring stimulant in *Ephedra vulgaris* ('Ma-Huang' or 'herbal ecstasy') was the first substance synthesised in 1885, followed by amfetamine in 1887[2] and, subsequently, by several other phenethylamines. Massive misuse of amfetamine-type stimulants to prevent fatigue and maintain alertness during World War II spread to Japan in the post-war period and to other European countries (e.g. Sweden) as well as to Canada and the US in the 1960s.[3] After recognition of the alarming increase in amfetamine abuse and that huge quantities were diverted to the illicit market, amfetamine and related substances were included amongst Schedule II and III drugs of the Controlled Substances Act (Title II of the comprehensive Drug Abuse Prevention and Control Act of 1970).[4]

Since the mid-1980s, a new group of synthetic amfetamines, the methylenedioxy derivatives, have gained popularity as recreational drugs.[5] These psychomotor stimulants (described as 'entactogens') have behavioural effects similar to those elicited by amfetamines and hallucinogens.[6,7]

An increase in the use of amfetamine-type stimulants has been one of the most outstanding worldwide trends in drug abuse during the past years,[8] partly due to a progressively high consumption of methylenedioxy- and methoxy-substituted amfetamines among youth. Moreover, the use of classical amfetamines continues to be highly prevalent in some areas of the world.[9]

At the present time, as many as 14 metabolic precursors of amfetamine and metamfetamine are on the market.[10] In the EU, amfetamine-like appetite suppressants have been withdrawn from the market because of their toxicity. On the other hand,

the therapeutic value of some amfetamine-type stimulants, such as selegiline and methylphenidate, is well recognised.[11]

Regulations for urine drug testing require that ingestion of therapeutically prescribed drugs must be excluded before reporting positive findings for amfetamines. However, to discriminate between therapeutic prescription of precursor drugs and abuse of amfetamines, a sound knowledge of amfetamine metabolism and enantiomeric disposition is needed.[12] Although drug testing of amfetamines has mainly concentrated on analytical methods in standard biological substrates, especially urine samples,[13] the need for non-invasive (physically and ethically) procurement of biological matrices for clinical purposes and eventual application in toxicology has prompted research on the detection of amfetamine-type stimulants in non-conventional matrices, such as saliva, sweat and hair.[14]

This article reviews the clinical pharmacokinetics of amfetamine and amfetamine-related substances with psychostimulant activity in humans. Amfetamine-like hallucinogens structurally related to mescaline have already been extensively reviewed[15] and have been excluded. A systematic overview of the pharmacokinetic parameters of classical amfetamine-like compounds (amfetamine-related compounds and substances giving rise to amfetamine or metamfetamine through hepatic biotransformation) is presented. Recent data on the pharmacology and clinical pharmacokinetics of methylenedioxy derivatives and other newly emerging designer drugs, such as paramethoxyamfetamine (PMA) and 4-methylthioamfetamine (4-MTA), are detailed and compared with those from the prototype compounds amfetamine and metamfetamine. Finally, the implications of the pharmacokinetics of amfetamine-type stimulants in conventional and non-conventional biological matrices (e.g. saliva, hair, sweat, nails) for drug testing in clinical and forensic toxicology are described.

1. Chemical Structure and Properties of Amfetamine and Related Substances

Amfetamines show sympathomimetic and CNS stimulant activity.[1] The various types of amfetamine-related compounds are shown in table I.

As shown in figure 1 and figure 2, the basic structure of these compounds is a β-phenylisopropylamine group, which in some cases includes a primary amine (e.g. amfetamine, phentermine and PMA), and in the others a substituted amine (e.g. metamfetamine, selegiline and clobenzorex). Methylenedioxy derivatives have a methylenedioxy group forming a five-membered ring including C-3 and C-4 of the benzene ring. Finally, PMA and 4-MTA are synthetic compounds presenting the same structure as amfetamine but with a methoxy or a methylthio group in the C-4 position. With regard to the effect on the aromatic structure, the introduction of electron-withdrawing groups in the ring, such as a halogen (chlorphentermine and fenfluramine) results in a reduction of psychomotor stimulant effects but maintaining appetite suppressant effects.[56,57]

The length of the side chain is essential. Shortening or lengthening the chain greatly alters locomotor stimulation and neuronal norepinephrine uptake.[58] The methyl group on the α-carbon (R_2) confers resistance to oxidative deamination and, therefore, an increased metabolic half-life, whereas the introduction of a further methyl group, as for phentermine and derivatives, gives an additional protection against deamination. Shifting the α-methyl group to the β-carbon abolishes the typical stimulating effects of amfetamine.[59] On the other hand, the presence of an ethyl group in the α-position, as for *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), makes the compound a selective serotonin releasing agent with few or no dopaminergic effects and blunted hallucinogenic properties. Indeed, both α-ethylation and *N*-alkylation have been shown to diminish the hallucinogenic activity of the dimethoxyphenyl derivatives.[6]

The large majority of these substances have a chiral centre at the α-carbon (figure 1) with a pair of enantiomers,[60] although the racemic mixture is usually consumed. The enantiomers may show different pharmacological activity and body disposition.[31] For example, the (*S*)-(+)-isomer of amfetamine is a more potent CNS stimulant than the (*R*)-(−)-isomer, and the (*S*)-isomers of 3,4-methylenedioxymetamfetamine (MDMA) and 3,4-methylenedioxyethylamfetamine (MDE) are responsible for the psychostimulant and entactogenic activities as compared with the hallucinogenic

Table I. Classification of the amfetamine-type stimulants

Drug	Abbreviation, street name(s), comments	References
Classical amfetamines		
Amfetamine		2
Metamfetamine [(*S*)-(+)-metamfetamine] and its racemate [(*R,S*)-(±)-metamfetamine]	Speed, ice, crystal, crank, meth	16,17
Dexamfetamine [(*S*)-(+)-amfetamine]		18
Substances giving rise to amfetamine or metamfetamine through hepatic biotransformation		
Selegiline		12,19,20
Fenproporex		21,22
Benzfetamine		23-25
Furfenorex		23-25
Mefenorex		26
Fenetylline		27,28
Clobenzorex		29,30
Ethylamfetamine		12,31
Amfetaminil		12
Fencamine		12
Famprofazone		32-34
Phenylpropanolamine		35,36
Mesocarb		31
Prenylamine		37
Amfetamine-like agents		
Ephedrine		38,39
Cathine and cathinone	Obtained from *Catha edulis* or Khat	8
Pemoline		40
Methylphenidate		41-43
Phenmetrazine		44
Phendimetrazine		45
Amfepramone (diethylpropion)		46
Fenfluramine		47
Aminorex		48
Phentermine		49
Chlorphentermine		50
Methylenedioxy amfetamine derivatives ('designer drugs')		
3,4-Methylenedioxyamfetamine	MDA, tenamfetamine, love drug, love pill	51
3,4-Methylenedioxymetamfetamine	MDMA, ecstasy, XTC, Adam	52
3,4-Methylenedioxyethylamfetamine	MDE, MDEA, Eve	53
N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine	MBDB	6
Methoxy amfetamine derivatives and other compounds with hallucinogenic properties		
Methyl-2,5-dimethoxyamfetamine	DOM, serenity-tranquillity-peace, STP	15
2,5-Dimethoxy-4-bromoamfetamine	DOB, bromo-DMA	15
2,5-Dimethoxy-4-bromophenethylamine	2C-B, nexus, bromo, toonies	15
Paramethoxyamfetamine	PMA	54
4-Methylthioamfetamine	4-MTA	55

properties of the (*R*)-isomers.[61] Stereoselective metabolism for amfetamine,[62] metamfetamine[63] and MDMA[64] has been reported.

Amfetamine and related compounds are weak bases with a pKa around 9.9, a low molecular weight, a low protein binding (around 20%) and a moderately high volume of distribution.[65-73] These properties confer easy diffusion across cell membranes and lipid layers and to tissues or biological matrices with a more acidic pH than blood.[74] The

principal physicochemical and pharmacokinetic characteristics of classical amfetamine-type stimulants and substances giving rise to amfetamine or metamfetamine through hepatic biotransformation are shown in table II. Amfetamine-related designer drugs (methylenedioxy derivatives, PMA and 4-MTA) are also weak bases, with a molecular weight around 200 g/mol, and show pharmacokinetic characteristics similar to those of the classical compounds.

2. Mechanism of Action and Pharmacological Effects of Amfetamine, Metamfetamine and Designer Drugs

2.1 Mechanism of Action

Amfetamines are indirect monoamine agonists, producing release from presynaptic terminals of norepinephrine, dopamine and serotonin (5-hydroxytryptamine) in the CNS and at the peripheral level.[75,76] They interact with the membrane transporters involved in neurotransmitter reuptake and vesicular storage systems. It seems that amfetamines can be transported into the nerve terminals by passive entrance or through a reuptake transporter, thus acting as inhibitors of the reuptake of monoamines. Once inside the neuron, amfetamines reverse the direction of the membrane transporter, facilitating the efflux of norepinephrine, dopamine and serotonin to the synaptic cleft. In addition, they are mild inhibitors of the enzymes monoamine oxidase (MAO) A and B. Repeated administration of amfetamine-type stimulants depletes the catecholamine supply, producing a decrease in pharmacological effects (acute tolerance).

Metamfetamine, fenfluramine and the methylenedioxy derivatives (3,4-methylenedioxyamfetamine [MDA], MDMA and MDE) can inhibit the activity of the rate-limiting enzymes of dopamine or serotonin biosynthesis. Metamfetamine inhibits the activity of tyrosine hydroxylase and decreases the formation of dopamine, and MDMA and fenfluramine inhibit the activity of tryptophan hydroxylase and decrease the formation of serotonin.[77] Similar mechanisms have been recently reported for 4-MTA.[78]

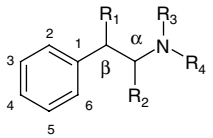
2.2 Pharmacological Effects

Amfetamines are powerful psychostimulants, producing increased alertness, wakefulness, insomnia, energy and self-confidence in association with decreased fatigue and appetite, as well as enhanced mood, well-being and euphoria. High doses can cause convulsions, stereotypic movements and psychosis. Fatigue, anxiety and tiredness can appear when the effects vanish.[79,80]

These negative symptoms ('crash') are more intense when high or repeated doses are administered, and depression and lethargy can appear. Long-term use of amfetamines may be associated with the so-called 'amfetamine psychosis' characterised by psychotic reactions, hallucinations and paranoia. Amfetamines show a high abuse potential and can induce dependence, tolerance and withdrawal symptoms.[81]

The effects of amfetamines at the peripheral level include systolic and diastolic blood pressure increase, mydriasis, tremor, sweating, jaw clenching, dry mouth and restlessness. These actions seem to be mediated by the release of norepinephrine, causing indirect sympathomimetic stimulation. Heart rate can be augmented (MDMA or methylphenidate) or remain unchanged due to a counteracting mechanism related to increase in blood pressure (amfetamine).[81,82]

The psychological effects of methylenedioxy derivatives seem to differ from those of the other amfetamines. MDMA users have consistently reported feelings of euphoria, happiness, increased energy, increased peacefulness, feeling close to others and increased insight, effects described by some authors as 'entactogenic'. Because of these effects, MDMA, MDA, MBDB (*N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine) and MDE are also known as 'entactogens'. Some authors have advocated the possible usefulness of MDMA for psychotherapeutic procedures. One important limitation to its therapeutic use is the risk of associated neurotoxicity.[6] The methoxy derivatives induce hallucinations at the usual recreational doses (see classification in table I).



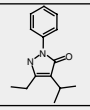
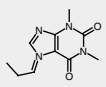
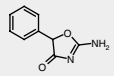
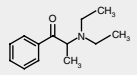
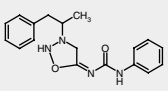
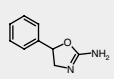
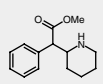
	R_1	R_2	R_3	R_4
Amfetamine	H	CH_3	H	H
Metamfetamine	H	CH_3	H	CH_3
Ethylamfetamine	H	CH_3	H	C_2H_5
Ephedrine	OH	CH_3	H	CH_3
Phenylpropanolamine	OH	CH_3	H	H
Selegiline	H	CH_3	CH_3	$CH_2C{\equiv}CH$
Phentermine	H	$(CH_3)_2$	H	H
Chlorphentermine	H	$ClCH_3$	H	CH_3
Fenproporex	H	CH_3	H	$(CH_2)_2CHCN$
Mefenorex	H	CH_3	H	$(CH_2)_3Cl$
Clobenzorex	H	CH_3	H	
Benzfetamine	H	CH_3	CH_3	
Amfetaminil	H	CH_3	H	
Furfenorex	H	CH_3	CH_3	
Famprofazone	H	CH_3	CH_3	
Fenetylline	H	CH_3	CH_3	
Fencamine	H	CH_3	H	
Fenfluramine*	H	CH_3	H	CH_2CH_3
Pemoline				
Amfepramone				
Phenmetrazine				
Phendimetrazine				
Mesocarb				
Aminorex				
Methylphenidate				

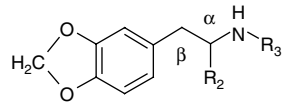
Fig. 1. Chemical structure of the main amfetamine-related substances. * = with a CF_3 group at C-4 of the benzene ring.

3. Drug Interactions and Toxicity

3.1 Drug Interactions

There is evidence for the safety of psychostimulants used in therapeutics (methylphenidate, dexamfetamine and selegiline) with most classes of medications, with a few absolute contraindications.[83] The interactions of abused stimulants with ethanol lead to relevant pharmacodynamic and mild pharmacokinetic interactions. The deleterious effects of ethanol on psychomotor performance can be partially antagonised by coadministration of dexamfetamine.[84] For metamfetamine, increased cardiac work was observed in the presence of ethanol,[85] and metamfetamine metabolism appeared to be affected by the drinking habits of subjects.[86] The interaction between MDMA and ethanol leads to a dissociation between subjective and objective sedation. Subjects may feel euphoric and less sedated and may have feelings of better performance, but actual performance ability continues to be impaired by the effect of alcohol.[87] There have been several reports on the manipulation of MDMA preparations by spiking with dextromethorphan.[88] Because both drugs are substrates of cytochrome P450 (CYP) 2D6, a pharmacokinetic interaction has been anticipated. No clinical cases have been reported, suggesting that such interaction has little toxicological impact. Antiretroviral drugs have been reported as the main cause of life-threatening interactions with MDMA.[89]

A number of clinical trials have been conducted to characterise MDMA pharmacology in humans, using citalopram, haloperidol and ketanserin as pharmacological challenges. Liechti et al.[90] found that pretreatment with intravenous citalopram 40mg significantly reduced the psychological and physiological effects of MDMA, but the effect lasted longer (mean 5 hours). Citalopram reduced the cardiovascular response and acute vegetative effects of MDMA.[91] Pretreatment with intravenous haloperidol 1.4mg significantly reduced MDMA-induced positive mood and euphoria, but had no effect on cardiovascular stimulation.[92] In another study,[93] pretreatment with oral ketanserin 50mg significantly reduced MDMA-induced perceptive changes, emotional excitability and vigilance. The



	R_2	R_3
MDA	CH_3	H
MDMA	CH_3	CH_3
MDE	CH_3	CH_2CH_3
MBDB	CH_2CH_3	CH_3

4-MTA **PMA**

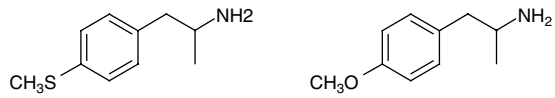


Fig. 2. Chemical structure of methylenedioxy and methoxy derivatives of amfetamine. **MBDB** = *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine; **MDA** = 3,4-methylenedioxyamfetamine; **MDE** = 3,4-methylenedioxyethylamfetamine; **MDMA** = 3,4-methylenedioxymetamfetamine; **PMA** = paramethoxyamfetamine; **4-MTA** = 4-methylthioamfetamine.

pharmacokinetic interactions of these drugs with MDMA were not investigated, nor have clinical case studies been reported in the literature, except for citalopram.[91]

In general, amfetamines and other amfetamine-like appetite suppressants, other than fenfluramine, would be expected to impair the hypotensive effects of adrenergic neuron-blocking drugs, such as guanethidine. Not only do they release norepinephrine from stores in adrenergic neurons and block the reuptake of released norepinephrine into the neuron, but they also impair reentry of the antihypertensive drugs. Amfetamines should not be used together with, or within 2 weeks of, any MAO inhibitors; severe hypertensive reactions and confusional states can occur. Amfetamine, metamfetamine and methylphenidate can enhance the analgesic effects of opioids and, finally, tricyclic antidepressants increase blood concentrations of amfetamine because of a metabolic interaction.[94]

3.2 Adverse Effects and Overdose

The acute toxic effects of amfetamines are usually related to their pharmacological actions.[65] Dexfenfluramine and fenfluramine were withdrawn from the market because of association with valvu-

Table II. Main physicochemical and pharmacokinetic properties of classical amfetamines and related substances

Compound	Therapeutic indication	MW	pK_a	Vd (L/kg)	f_b (%)	Active metabolites	$t_{1/2\beta}$ (h)	Ae (%)	References
Amfetamine	Psychostimulant	135.2	9.9	3–4	16		12, 4–8[a]	30	65,66
Metamfetamine	Psychostimulant	149.2	9.9	3–7	10–20			70	65,66
Dexamfetamine	Psychostimulant	135.2							66
Ethylamfetamine	Appetite suppressant	163.2				AM		22.2–54.4	11,31
Ephedrine	Bronchodilator, psychostimulant	174.2	9.6				6	88	38,39,66-69
Phenylpropanolamine	Nasal vasoconstrictor, appetite suppressant	151.2	9.1	4.5		AM/MA	3.0–4.4	90	35,36,65-67
Selegiline	Antiparkinsonian	187.3	6.8	4.3	94	AM/MA	39	50	12,19,65,66,69
Phentermine	Appetite suppressant, psychostimulant	149.2	10.1	3–4			19–24	48, 84[a]	49,66,67,71
Chlorphentermine	Appetite suppressant	183.7	9.6	3.0			40	17. 7[a]	50,65-68
Fenproporex	Appetite suppressant	188.3				AM/MA		5.4–8.7[a]	12,22,66,67
Mefenorex	Appetite suppressant	211.7				AM/MA		1	12,66,70
Clobenzorex	Appetite suppressant	259.8				AM		30	12,30,66,70
Benzfetamine	Appetite suppressant	239.4	6.5			AM/MA			12,24,66,67
Amfetaminil	Appetite suppressant	250.2							12
Furfenorex	Appetite suppressant	229.3				AM/MA		Trace amounts	12,24,70
Famprofazone	Analgesic, antipyretic	377.5				AM/MA			34,57,66
Fenetylline	Psychostimulant	341.4				AM		3.6	12,27,66,67
Fencamine	Psychostimulant	384.5				AM/MA		26.6	12,67,70
Fenfluramine	Appetite suppressant	231.3	9.9	12–16	34		13–30[a]	3–10[b] or 23[a,b]	65,66
Dexfenfluramine	Appetite suppressant	231.3		6.68			17.8		47,67
Pemoline	Psychostimulant	176.2	10.0	0.2–0.59	30		11	47.0	40,65,66,70
Amfepramone	Appetite suppressant	205.3					1.5–3	80–90	46,66,67
Phenmetrazine	Appetite suppressant	177.2	8.5				9.1	19, 46[a,c]	44,65,67,72,73
Phendimetrazine	Appetite suppressant	191.3	7.6				9.1	9[a]	45,70,72,73
Mesocarb	Psychostimulant	324.3				AM		Trace amounts	12,67
Aminorex	Appetite suppressant	162.2							48,67,70
Methylphenidate	Psychostimulant	233.3	8.8	11–33	15		2–3	80	41-43,65-67

a Acidic urine.

b In 48 hours.

c In 16 hours.

Ae = amount excreted in 24-hour urine collection; **AM** = amfetamine; $\mathbf{f_b}$ = fraction bound to protein; **MA** = metamfetamine; **MW** = molecular weight; $\mathbf{t_{1/2\beta}}$ = terminal elimination half-life; **Vd** = volume of distribution.

lar heart disease.[47,48] Amfetamines with appetite suppressant activity have been associated with an increased risk of pulmonary hypertension.[57] Typical signs of mild toxicity include nausea, vomiting, mydriasis, dry mouth, sweating, hyperreflexia, bruxism, trismus and palpitations. Moderate intoxications can include hyperactivity, anxiety, confusion, panic attack, psychosis with hallucinations, tachycardia, hypertension and increased body temperature. Suicidal and homicidal tendencies can occur. Severe intoxication includes delirium, coma, seizures, hypotension, dysrhythmia, hyperpyrexia and renal failure associated with rhabdomyolysis. All amfetamines can induce acute ischaemic and haemorrhagic stroke.

One important factor in MDMA overdose is hyperthermia, which may result from a direct action of the drug on the temperature-regulating centre, from muscular activity associated with dance or tremor and rigidity, or from high ambient temperatures in crowded places and dehydration. Heat stroke is a severe complication that can cause death; it manifests as hyperpyrexia, rhabdomyolysis, myoglobinuria, disseminated intravascular coagulation and renal failure. Hyponatraemia is an uncommon complication that can be caused by drinking excessive amounts of water and/or by MDMA-induced secretion of antidiuretic hormone (syndrome of inappropriate secretion of antidiuretic hormone, or SIADH). Fulminant hepatitis has been also reported.[95]

3.3 Neurotoxicity

It has been extensively documented that chronic misuse of amfetamines may lead to long-lasting impairment of brain function.[96] Animal studies showing neurochemical and morphological changes in dopamine or serotonin neurons in response to the administration of metamfetamine, methylenedioxy analogues and fenfluramine/dexfenfluramine have partially been confirmed with brain imaging studies in humans (reductions in dopamine/serotonin transporters).[97-104] Although neurotoxicity to monoaminergic systems seems a class property of amfetamines, there is some selectivity, for example amfetamine and metamfetamine are characterised by long-term deficits of both dopamine and serotonin, whereas MDMA and fenfluramine primarily affect serotonin. In addition, MDMA-induced changes in catecholaminergic systems are mild and transient, and no long-term deficits of dopamine, norepinephrine and their metabolites or tryptophan hydroxylase activity have been reported.[77]

The mechanisms underlying neurotoxic effects are poorly understood, although a pathogenetic role of amfetamine-, metamfetamine- or MDMA-induced hyperthermia and reactive species (free radical) formation in brain regions has been postulated.[103] Neurotoxicity of methylenedioxy derivatives can be reduced or prevented by the administration of different substances that act at various levels, for example aspirin (acetylsalicylic acid), fluoxetine and citalopram or barbiturates. Hypothermia and drugs that decrease body temperature also reduce neuronal toxicity.[103,105]

4. Pharmacokinetics

Absorption, distribution and elimination of amfetamines, like other drugs, are dependent on their physical and chemical properties.[106] The kinetic parameters of amfetamine, metamfetamine and designer amfetamines (methylenedioxy derivatives and PMA) are summarised in table III.

4.1 Absorption

Amfetamines are consumed by different routes.[125] Amfetamine itself is most often consumed orally, either as the (*S*)-(+)-enantiomer (dexamfetamine) or the racemic mixture (i.e. amfetamine sulphate).[107,125] After a single oral dose, amfetamine is rapidly absorbed, with a mean maximum plasma concentration (C_{max}) within 4 hours after ingestion (t_{max}). Both the area under the plasma concentration-time curve in the 24 hours after administration (AUC_{24}) and C_{max} are proportional to the dose administered.[84,108,109] After the administration of both racemate and individual isomers of amfetamine, no significant differences between pharmacokinetic parameters of the two enantiomers during the absorption phase (C_{max} and t_{max}) have been documented.[110] Drug absorption seems to be affected by overnight fasting. This circumstance may be of practical importance when treating children with stimulants at the time of testing for potential hyperactivity syndrome.[109]

Table III. Pharmacokinetic parameters of classical and designer amfetamines (AMs). Data are presented as mean ± SD (range); doses are presented as base drug

Drug	Dose (mg)	Route	n	Isomer studied	C_{max} (μg/L)	t_{max} (h)	AUC_{24} (μg • h/L)	V_{ss} (L)	f_b (%)	$t_{½β}$ (h)	CL_R (L/h)	Ae (%)	References
AM[a]	20	Oral	1		36.6	3	482.5						108
	20	Oral	1		38.8	2	431.6						
	30	Oral	1		57.3	3	790.2						
	30	Oral	1		57.8	2	753.1						
	35	Oral	1		63.5	2	822.9						
	35	Oral	1		57.5	2	758.6						
	40	Oral	11		69.1 ± 5.7	2.2 ± 1.0	945.4 ± 71.8						
(*S*)-(+)-AM	0.25[b]	Oral (fasting)	7		35.3 ± 3.4	3							109
	0.25[b]	Oral	7		39.6 ± 2.8	3							
	0.50[b]	Oral	8		67.3 ± 5.4	4							
(*S*)-(+)-AM	0.06[b]	Oral	12		18.3 ± 1.4	1.9 ± 0.2	49.4 ± 3.4[c]						84
	0.10[b]	Oral	12		21.4 ± 0.7	2.5 ± 0.3	58.3 ± 2.3[c]						
AM	10[d]	Oral	4	(*S*)-	20[e]	4[e]		237.6 ± 26.9[f]		17.0 ± 1.5[f]			110
				(*R*)-	20[e]	4[e]		243.4 ± 29.0[f]		23.7 ± 3.5[f]			
AM	10[g]	Oral	4	(*S*)-	18[e]	4[e]		210.3 ± 51.3[f]		6.8 ± 1.0[f]			
				(*R*)-	17[e]	4[e]		248.1 ± 78.3[f]		7.7 ± 1.0[f]			
(*S*)-(+)-AM	10[d]	Oral	4		40[e]	2.5[e]		258.1 ± 32.7[f]	16	15.6 ± 1.3[f]			
(*R*)-(−)AM	10[d]	Oral	4		40[e]	3[e]		267.4 ± 38.1[f]	16	25.0 ± 2.3[f]			
(*S*)-(+)-MA	17.5	Smoked	6		50[e]	(1–2)[e]	1013.0 ± 141.0[f,h]	3.2 ± 0.4[b,f]		11.8 ± 1.4[f]	6.7 ± 0.8[f]	36.8 ± 4.3[h]	111
	12.4	Intravenous	6		90[e]	(1–2)[e]	787.0 ± 29.7[f,h]	3.7 ± 0.6[b,f]		13.1 ± 1.5[f]	6.9 ± 1.3[f,i]	45.0 ±9.5[hi]	
MA	17.7	Vapour inhalation	6		47.1 ± 5.6	2.5 ± 0.5							112
(*S*)-(+)-MA	24.1	Intravenous	8		140[e]	1.1[e]		304.0 ± 26.0		12.0 ± 3.2	7.1 ± 2.1	38.7 ± 10.1[i]	85
MA	10	Oral	6		23[e]	3.1 ± 0.3							113
(*S*)-(+)-MA	0.25[b]	Oral	8			3.2				11.4	8.28		114
(S)-(+)-MA[j]	0.34[b]	Oral (06.30)	5		94.1 ± 70.9 (61.8–291.4)	3.6 ± 0.6 (3.0–5.0)	599.1 ± 131.2 (399.6–859.5)[k]	4.6 ± 1.4 (1.4–6.4)[bl]		9.1 ± 4.0 (2.8–16.7)	0.15 ± 0.06[m] (0.07–0.25)	19.9 ± 6.5 (12.2–34.2)	17

Continued next page

Table III. Contd

Drug	Dose (mg)	Route	n	Isomer studied	C_{max} (μg/L)	t_{max} (h)	AUC_{24} (μg • h/L)	V_{ss} (L)	f_b (%)	$t_{½β}$ (h)	CL_R (L/h)	Ae (%)	References
	0.34[b]	Oral (18.30)	5		60.4 ± 16.9 (29.7–77.0)	4.9 ± 2.4 (2.5–10.0)	469.7 ± 121.5 (260.7–616.0)[k]	6.1 ± 2.6 (4.0–12.3)[b,l]		10.8 ± 7.9 (4.3–31.8)	0.22 ± 0.08[m] (0.13–0.34)	22.8 ± 7.3 (15.8–42.2)	
(*S*)-(+)-MA								3–7[b]		6–15			73
(*S*)-(+)-MA	50	Intranasal	8		113.0 ± 23.1	2.66 ± 1.16	2000 ± 599[h]			10.7 ± 2.39	102 ± 55.1	39.3 ± 24.5	115
(*S*)-(+)-MA	40	Smoked	8		50.9 ± 24.7	2.47 ± 3.91	801 ± 526			10.7 ± 2.11	98.9 ± 55.9	36.0 ± 17.7	115
(*S*)-(+)-MA*	10	Oral	8		20.2 ± 6.4 (14.5–33.8)	5.4 ± 2.5 (2.0–8.0)	269.1 ± 94.3 (84.0–357.2)	5.8 ± 2.6 (1.6–8.9)		9.3 ± 3.7 (2.1–14.0)	32.2 ± 13.7 (22.9–62.4)		116
(*S*)-(+)-MA*	20	Oral	5		32.4 ± 7.7 (26.2–44.3)	7.5 ± 3.4 (2.0–11.5)	468.1 ± 151.8 (258–621.3)	5.3 ± 2.1 (1.7–6.7)		11.1 ± 7.2 (2.2–21.2)	33.5 ± 15.9 (21.0–55.4)		116
MDMA	75	Oral	12		178 ± 52	(2.0–4.0)							117
MDMA	50	Oral	2		(19.8–82.2)	(2–3)	(100.1–813.9)		20	(2.7–5.1)	(73.3–4.9)	(8.0–15.8)	118,119
	75	Oral	8		130.9 ± 38.6	1.8 ± 0.4	1331.5 ± 646.0			7.7 ± 3.2	12.8 ± 5.6	18.3 ± 3.5	
	100	Oral	2		(189.9–209.7)	(2–3)	(1447.8–2256.6)			(5.8–8.5)	(12.3–20.4)	(14.4 –45.0)	
	125	Oral	8		236.4 ± 58.0	2.4 ± 1.0	2623.7 ± 572.9	452 ± 137		8.6 ± 3.2	13.0 ± 5.4	26.2 ± 10.7	
	150	Oral	2		(441.9–486.9)	(1.5–2)	(5132.8–5232.0)			(6.9–7.2)	(5.2–11.3)	(20.6–43.0)	
MDMA	1.5[b]	Oral	2		331	2							120
MDMA	100	Oral	8		181.4 ± 31.3	1.5	1598.6 ± 733.3			7.2 ± 1.4			121
MDMA	40	Oral	8	(*R*)-	33.7 ± 14.9	4		383 ± 97		5.8 ± 2.2	10.5 ± 2.9		122
				(*S*)-	21.2 ± 10.8	2		595 ± 204		3.6 ± 0.9	10.2 ± 3.4		
MDE	118.6	Oral	6		260.0 ± 45.2 (203–333)	2.1 ± 0.5 (1.6–2.9)	(657–875)[c]						123
	1.7[b]	Oral	8		332 ± 68.4 (235–465)	2.9 ± 1.1 (110–300)	(832–1935)[n]						
MDE	59	Oral	5	(*R*)-	127.8 ± 34.2	2.8 ± 0.9	1706.8 ± 896.9[o]			7.5 ± 2.4	43.1 ± 18.0[p]		61
				(*S*)-	80.0 ± 29.5	2.6 ± 0.6	535.4 ± 262.5[o]			4.2 ± 1.4	135.6 ± 61.9[p]		
PMA	50–100	Oral			(200–400)[e]							15	54,65
4-MTA										7[e]			124

a For AM, MA and MDMA, dextro (D-) configuration is represented as the (*S*)-(+)-enantiomer [D- = (*S*)-(+)- = (+)-]. When configuration is not reported, administration of the racemate should be assumed.[107]

b Value expressed per kg bodyweight.

c Parameter measured from 0 to 4 hours.

d Administered with $NaHCO_3$.

e Approximate value (e.g. derived from a graph).

f Values are expressed as mean ± standard error.

g Administered with NH_4Cl.

Continued next page

Table III. Contd

h	Parameter measured from 0 to infinity.
i	Parameter measured from 0 to 48 hours.
j	Pharmacokinetic parameters calculated in serum instead of plasma.
k	Parameter measured from 0 to 12 hours.
l	Apparent steady-state volume of distribution.
m	Data expressed in L/h/kg.
n	Parameter measured from 0 to 6 hours.
o	Parameter measured from 0 to 34 hours.
p	Total clearance.

4-MTA = 4-methylthioamfetamine; **Ae** = amount excreted in 24-hour urine collection; **AM** = amfetamine; $\mathbf{AUC_{24}}$ = area under the plasma concentration-time curve from 0 to 24 hours after administration; $\mathbf{CL_R}$ = renal clearance; $\mathbf{C_{max}}$ = peak plasma drug concentration; $\mathbf{f_b}$ = fraction bound to protein; **MA** = metamfetamine; **MDE** = 3,4-methylenedioxyethylamfetamine; **MDMA** = 3,4-methylenedioxymetamfetamine; **n** = number of patients;**PMA** = parametoxyamfetamine; $\mathbf{t_{max}}$ = time to C_{max}; $\mathbf{t_{1/2\beta}}$ = terminal elimination half-life; $\mathbf{V_{ss}}$ = volume of distribution at steady state; * = sustained release formulation.

In contrast to amfetamine, metamfetamine can be taken/misused by the oral route, injected intravenously or snorted ('speed'). Smoking (*S*)-(+)-metamfetamine hydrochloride ('ice') through a glass pipe system is increasingly common among users.[126] Smoking of the drug is generally followed by a rapid onset of action (comparable with cocaine after intravenous use).[127] In fact, the pharmacokinetics of smoked metamfetamine hydrochloride in six volunteers showed parameters rather similar to those after intravenous injection in the same subjects. In addition, smoked metamfetamine showed an excellent bioavailability (90%), markedly higher than after drug use by the oral route (67%).[111] Other pharmacokinetic data on smoked (vapour inhalation) and intravenous administration of metamfetamine confirmed the rapid rise of plasma drug concentrations, with t_{max} values between 1 and 2.5 hours after drug use, and sustained plasma concentrations for approximately 4 hours.[85,112,115] In contrast, oral administration of metamfetamine results in a delay in reaching C_{max}, appearing at least 3 hours after the dose, which is in agreement with the t_{max} of amfetamine after oral intake.[113,116] In general, no differences in day versus night pharmacokinetic profiles of (*S*)-(+)-metamfetamine after oral administration have been observed. Despite the fact that C_{max} in night-time assessments was approximately 55% lower than that for daytime, there was a marked between-subject variability and no significant differences were found.[17] Furthermore, the mean pharmacokinetic data obtained in this study were in close agreement with those reported after oral metamfetamine 0.25 mg/kg.[114]

Methylenedioxy derivatives and 'designer drugs' such as 4-MTA and PMA are mostly consumed orally but, in contrast to amfetamine and metamfetamine, the racemic mixture is usually ingested.[128]

No pharmacokinetic data are available for MDA in humans. This drug used to be popular at the end of 1960s as 'love pill', but at the present time it is mostly used for MDMA synthesis.[51] Evidence available in the literature is based on animal models and intoxication data.[129] After oral ingestion of MDMA, C_{max} appears at 1.5–3 hours, which is consistent with data obtained for the prototype compounds. It should be noted that after the administra-

tion of five different doses of MDMA (50, 75, 100, 125 and 150mg), C_{max} and AUC_{24} both increased according to the dose given,[118,119] although for the 150mg dose the increase in MDMA kinetic parameters was not proportional to the dose, suggesting nonlinear pharmacokinetics. This finding was explained by a possible saturation of MDMA metabolism as well as by interaction of MDMA metabolites in some of their metabolic pathways. *In vitro* data suggest that MDMA can act as inhibitor of CYP2D6, through mechanisms that include a competitive interaction and/or the formation of a metabolic complex between MDMA and this enzyme.[118] Pharmacokinetic parameters reported by others[117,120,121] using both similar and different MDMA doses are consistent with these findings.[118]

An enantioselective disposition of MDMA has been described in humans. After an oral administration of 40mg of racemic MDMA, the mean C_{max} of (*R*)-(−)-MDMA was significantly greater than that of (*S*)-(+)-MDMA, and the mean enantiomeric ratio (*R*/*S*) of AUC_{24} indicated a more rapid elimination of the more pharmacologically active (*S*)-(+)-enantiomer.[64,122] Although there is a study reporting excretion of MBDB and its demethylated metabolite 3,4-(methylenedioxyphenyl)-2-butanamine (BDB) following single oral administration, the drug has not been recovered or quantified in plasma samples.[130]

MDE is another designer drug with a similar acute psychoactive profile to that of MDMA but with less neurotoxic effects at equivalent doses.[131] The pharmacokinetics of MDE when given orally are similar to those of MDMA (e.g. C_{max} 203–465 μg/L, for 118.6mg or 1.7 mg/kg bodyweight, t_{max} 1.6–4 hours).[123] Enantiomers of MDE were analysed in plasma samples of individuals treated orally with a racemic mixture of the drug; C_{max} and AUC_{34} of (*R*)-(−)-MDE were significantly larger than those of the (*S*)-(+)-enantiomer, indicating a stereoselective disposition of MDE in humans, which is in agreement with data reported for MDMA.[61]

Absorption profiles of 4-MTA and PMA after controlled drug administration are unknown. However, there are a few studies on the identification of these drugs in biological fluids and tissue samples in cases of fatal and non-fatal intoxication.[54,124,132,133] In particular, it seems that moderate toxicity for 4-MTA may be associated with plasma concentrations of 200–600 μg/L, severe toxicity >600 μg/L and death >1500 μg/L.[133] For PMA, all deaths involved its oral administration in tablets containing 50–90mg and resulted in blood concentrations greater than 1000 μg/L. According to these findings, it is assumed that PMA may be more toxic than MDMA. Concentrations higher than 500 μg/L appear to be associated with toxic effects and may be lethal, especially in combination with other amfetamines.[59] On the other hand, it has been hypothesised that, by analogy with amfetamine and MDMA, hallucinogenic effects of PMA appear at plasma concentrations of 200–400 μg/L.[54,65]

4.2 Distribution

Amfetamines have, in general, a low protein binding (<20%) [table II and table III].[125] In practice, this low binding means that almost the total amount of drug available in plasma may diffuse to the extravascular compartment. It has been suggested that amfetamine-dependent individuals have larger volume of distribution (e.g. 6.1 L/kg) than naïve individuals (3.5–4.6 L/kg) due to a higher tissue affinity for the drug in dependent subjects.[134,135] It appears, therefore, that amfetamine might be sequestered in tissue compartments as a result of development of tolerance. A longer plasma elimination half-life in drug-dependent subjects than in non-users (21.8 ± 1.4 vs 13.9 ± 3.4 hours in alkaline urine) at the same 25mg oral dose further supports the tolerance phenomenon.[136] Since metabolism was shown not to be different between tolerant and non-tolerant individuals, the results are indicative of a higher distribution volume and, thus, higher amfetamine tissue affinity in tolerant amfetamine abusers.[137] Concerning the distribution of the two amfetamine isomers, it has been shown[110] that protein binding and distribution volumes of (*S*)-(+)- and (*R*)-(−)amfetamine enantiomers are similar, whereas the elimination half-life of (*S*)-(+)-amfetamine was shorter than that of the (*R*)-(−)-isomer because of the faster metabolism of the more pharmacologically active enantiomer.[109]

The volume of distribution of metamfetamine appeared to be similar to that of amfetamine and unaffected by the route or time of administra-

tion.[17,85] Concurrent use of ethanol significantly decreased the volume of distribution of metamfetamine. This fact was explained by ethanol possibly displacing metamfetamine from peripheral binding sites.[85] Information on the distribution parameters of designer amfetamines in humans after controlled administration is very limited. From the data reported by de la Torre et al.,[118] a distribution volume of 452 ± 137L (6.4 L/kg) can be established following oral administration of MDMA 100mg. With regard to MDMA enantiomers, Fallon et al.[122] indicated the more extensive distribution of the more active (*S*)-(+)-MDMA enantiomer, supporting the hypothesis of enantioselective disposition of MDMA in humans.

4.3 Distribution and Accumulation in Other Biological Matrices

Little attention has been paid, in the past, to minor excretion pathways of drugs (e.g. tears, saliva and sweat) as well as their accumulation in some tissues (e.g. hair and nails). From a quantitative perspective, they are supposed to contribute little to the overall drug disposition process. The passage of lipid-soluble compounds from blood to other fluids or matrices is regulated by their pKa and the pH in each biological fluid, interrelated by a modified version of the Henderson-Hasselbach equation, which allows theoretical calculation of the fluid to plasma concentration ratio (F/P ratio).[138] Because amfetamines are weak bases with a generally low protein binding, these compounds tend to accumulate in biological fluids and matrices with pH values lower than that of the plasma (pH 7.4) ['ion-trapping phenomenon']. Therefore, given that saliva and sweat are both more acidic than plasma, it is apparent that amfetamines may be readily distributed in these body fluids.

Accumulation of amfetamines in a keratin matrix (hair and nails) depends on complex mechanisms not fully understood. In the case of hair, hypothesised factors include:[139]

- transport across the membranes;
- biotransformation and drug melanin affinity, depending on the physicochemical properties of the drug, such as lipid solubility, molecular size and geometry of the drug molecule;
- concentration gradient;
- pH gradient;
- plasma protein binding; and
- blood flow at hair follicles.

The axial distribution of drugs along the hair length is supposed to represent approximately the month-by-month dosage history.[74]

In contrast with hair, nails do not contain melanin and this may reduce drug incorporation into this substrate. However, studies with antifungal drugs suggest incorporation from the systemic circulation via nail bed and nail matrix.[140] This double mechanism of incorporation does not allow temporal evaluation of drug intake, such as can be obtained from hair samples.[141]

4.3.1 Saliva

Experimental values of saliva to plasma (S/P) ratio always exceed the theoretical value (table IV), showing high accumulation of amfetamine-type drugs in this biological matrix.

Discrepancies between S/P ratios predicted by the modified equation of Henderson-Hasselbach and experimental observations have been explained in some cases by the possibility of oral cavity contamination for drugs ingested orally or smoked.[111] However, the few experiments where amfetamines were administered intravenously, as well as analysis of data from post-absorption phases where oral contamination can be excluded, have consistently documented higher ratios than predicted. The pharmacology of these drugs and haemodynamic changes can be at the origin of these observations.[82] The second source of variability to be taken into account is the procedure followed for the procurement of saliva samples. Working with samples where saliva production was stimulated (chewing) or non-stimulated (spitting of natural saliva production) may lead to different results because of changes in salivary pH.[157]

After oral administration of both the racemate and the individual isomers of amfetamine, drug salivary concentrations, as for plasma, peaked between 2 and 4 hours.[109] However, the amfetamine concentration found in saliva was at least twice that in plasma, exceeding the theoretical S/P ratio during the absorption phase. The (*S*)-(+)- and (*R*)-(−)-enantiomers were similarly absorbed, whereas the (*S*)-

(+)-enantiomer was the more rapidly eliminated (similar to plasma).[110] The results obtained were consistent with the concentration of amfetamine found in the saliva of some habitual consumers (range 40−60 μg/L).[142]

Although the secretion of metamfetamine from blood into saliva has been investigated by some authors,[111,155,158] only one study with (*S*)-(+)-metamfetamine smoked and injected intravenously reported salivary concentrations after administration. Saliva concentrations immediately after smoking were very large because of oral contamination. However, in the post-absorption phase, S/P ratios, although showing great variability, indicated significant accumulation of metamfetamine in saliva, as was also observed for (*S*)-(+)-metamfetamine injected intravenously.[111]

The pharmacokinetics of MDMA in saliva have been reported after controlled oral administration of 75 and 100mg doses.[117,121] The experimental S/P ratio greatly exceeded the theoretical value, showing accumulation of MDMA in saliva. Presumably MDMA impairs salivary flow through its sympathomimetic effects, producing a sympathetic constriction of the salivary bed. Consequently, buffering capacity, which is maximal in conditions of flow stimulation, will be reduced and the pH of mixed saliva obtained from the oral cavity (as measured) may not be the same as the pH at the site of saliva secretion. Thus, a dynamic concentration gradient occurs, which probably produces MDMA S/P ratios higher than those calculated with the Henderson-Hasselbach equation.

Salivary concentrations of MDMA obtained after controlled administration of the drug were consistent with those obtained by Samyn et al.[143] in MDMA consumers undergoing roadside drug testing. In consumers, these authors also reported concentrations of 15−12 585 μg/L for amfetamine, 14−264 μg/L for MDA and 4−268 μg/L for MDE.[14,143]

The pharmacokinetics of MBDB in saliva showed similar patterns to those of MDMA. After administration of a 100mg oral dose of MBDB to one subject, a peak salivary concentration of 1 mg/L was found at 2 hours.[130] If MBDB plasma concentrations are of the same magnitude as for MDMA, a significant accumulation in saliva may also be expected for this methylenedioxy derivative.

4.3.2 Sweat

Excretion of amfetamines in sweat was first reported in 1972.[159] A non-stimulating derivative, (*S*)-(+)-dimethylamfetamine, was administered as a 20mg oral dose in two volunteers. Sweat was collected under physical exercise stimulation for 54 hours. Maximum (*S*)-(+)-dimethylamfetamine concentration, of the order of 2000−4000 μg/L, was found at 1.5 hours after administration. The metabolite of (*S*)-(+)-dimethylamfetamine, (*S*)-(+)-metamfetamine, was also found, with maximum concentrations of 330 and 750 μg/L at 5.5 and 7 hours post-administration, respectively.

Although the use of sweat for drug testing has been hampered by difficulties in sample recovery and sensitivity of analytical methods,[160] significant advances facilitating sample collection and improving accuracy of diagnostic techniques have permitted successful sweat testing for several drugs of abuse. These include the development of the sweat patch technology.[144,161,162]

Similarly to saliva, sweat has a pH (mean 6.3) lower than that of plasma, so that the free fraction of drug (unbound to proteins) diffuses through lipidic membranes by passive diffusion.[163]

For this reason, several authors expected an accumulation of amfetamine derivatives in sweat and monitored this biological matrix in suspected consumers. Amfetamine-type compounds such as metamfetamine, MDA, MDMA and MDE were found to appear in potential abusers wearing sweat patches, at concentrations ranging from tens to hundreds of nanograms per patch.[14,145,162,164]

Two controlled administration studies reported the accumulation of MDMA and MBDB in sweat patches.[130,163] In the first study, MDMA was already detected in the sweat patches at 1.5 hours after consumption, showed a first maximum value at 6 hours (229.3−337.0 ng/patch), showed an inflexion at 10 hours due to skin reabsorption and peaked again at 24 hours (table IV). Conversely, when using a cotton wipe instead of a patch to collect sweat in a controlled administration of 75mg MDMA, concentrations never exceeding 25ng MDMA/wipe were observed within five hours after administration.[117]

Table IV. Accumulation of classical and designer amfetamines (AMs) in the principal non-conventional biological matrices. Data are presented as mean ± SD (range)

Drug	Dose (mg)	Route	n	Isomer studied	Theoretical F/P ratio[a]	Experimental F/P ratio	C_{max} (μg/L)	t_{max} (h)	Reference
Saliva									
AM	10[b]	Oral	4	(*S*)-	2.2	2.1–3.8	50[c]	4[c]	110
				(*R*)-		2.2–4.1	45[c]	4[c]	
AM	10[d]	Oral	4	(*S*)-		2.0–3.6	55[c]	4[c]	
				(*R*)-		2.0–3.8	55[c]	4[c]	
(*S*)-(+)-AM	10[b]	Oral	4				110[c]	4[c]	
(*R*)-(–)-AM	10[b]	Oral	4				120[c]	2[c]	
AM							(40–60)		142
AM							(15–12585)		143
(*S*)-(+)-MA	21.8	Smoked	6		4.0	5.1[e]	9500[c]	1[c]	111
	15.4	Intravenous	6			6.0	500[c]	1[c]	
MDMA	100	Oral	8		3.9	18.1 ± 7.9[f]	3375.6 ± 812.8	1.5	121
MDMA		Oral	9			(1.0–16.4)	(218–6280)		14
MDA		Oral	8				(14–264)		14
MBDB	100	Oral	1				510		14
MBDB		Oral	1				1083.0	2	130
MDE		Oral	6				(4–268)		14
MDMA	75	Oral	12		3.9	12 ± 6	1215 ± 944	2–4	117
Sweat									
MDMA		Oral	4[g]				237 (138–431)[h]		14
MDMA	100	Oral	9				542.1 ± 433.5 (42.5–1326.1)[h]	24	144
MBDB	100	Oral	1				44[h]	36	122
MDE		Oral	3[g]				232 (171–281)[h]		15
MDE		Oral	1				121[h]		145
MDMA	75	Oral	12				25*		117
Hair									
AM			5				0.46 ± 0.19 (0.03–1.2)[i]		146
AM			1				10.2[i]		147
AM			23				0.8 (0.02–6.5)[i]		148
AM			20				(0.1–4.8)[i]		149
MA			7				3.67 ± 1.45 (0.1–10.2)[i]		146

Continued next page

Table IV. Contd

Drug	Dose (mg)	Route	n	Isomer studied	Theoretical F/P ratio[a]	Experimental F/P ratio	C_{max} (μg/L)	t_{max} (h)	Reference
MA							(0.5–15.6)[i]		150
MDMA		Oral	7				(0.2–12.5)[i]		151
MDMA			1				53.4[i]		147
MDMA		Oral	9				1.4 (0.05–2.9)[i]		148
MDMA		Oral	16				(0.1–8.3)[i]		150
MDMA		Oral	14				(0.3–42.7)[i]		149
MDA			1				8.0[i]		147
MDA			6				0.4 (0.04–1.2)[i]		148
MDA			13				(0.4–8.0)[i]		149
MBDB		Oral	2				(0.2–1.3)[i]		150
MBDB		Oral	2				(1.4–3.1)[i]		149
MDE			2				0.7[i]		151
MDE		Oral	5				1.5 (0.8–3.1)[i]		148
MDE		Oral	13				(0.1–15.0)[i]		150
MDE		Oral	6				(0.6–69.3)[i]		149
AM			1				0.7[i]		152
MA		Oral	5				(0.22–2.18)[i]		152
MDMA		Oral	14				(1.2–12.6)[i]		152
MDEA		Oral	1				0.7[i]		152
MDA		Oral	10				(0.15–9.0)[i]		152
Nails									
AM[j]			5				0.14 ± 0.06 (0.03–0.4)[k,l]		146
			3				0.6 ± 0.7 (0.03–1.6)[k,m]		
			3				(0.3–23.2)[k]		155
			1				12.0[k,l]		156
MA			9				4.7 ± 2.3 (0.32–17.7)[k,l]		156
			4				2.7 ± 4.1 (0.06–9.9)[k,m]		
			13				(0.4–642)[k]		155
MDA			1				9.7[k,l]		156
MDMA			1				60.2[k,l]		156

a Theoretical ratio obtained from the Henderson-Hasselbach equation.[121]

b Administered with $NaHCO_3$.

Continued next page

Table IV. Contd

c Approximate value derived from a graph.

d Administered with NH_4Cl.

e Value observed in the postabsorptive phase.

f Value observed at the maximum concentration time point.

g Volunteers worn a sweat patch for 28 hours during a weekend rave party. Non-controlled study.

h Value expressed as ng/patch.

i Value expressed as ng/mg of hair.

j AM was detected as MA metabolite.[153,154]

k Value expressed as ng/mg of nail.

l Concentration measured in fingernail.

m Concentration measured in toenail.

C_{max} = peak plasma drug concentration; **F/P** = fluid/plasma; **MA** = metamfetamine; **MBDB** = *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine; **MDA** = 3,4-methylenedioxyamfetamine; **MDE** = 3,4-methylenedioxyethylamfetamine; **MDMA** = 3,4-methylenedioxymetamfetamine; **n** = number of patients; **t_{max}** = time to C_{max}; * = value expressed as ng/wipe.

Intersubject variability was very large, as peak concentrations for the same dose varied 30-fold. Only traces of the minor metabolite MDA were detected.[163] In the second study, sweat was analysed in a subject wearing patches for 72 hours after consumption of a 100mg oral dose of MBDB. Both the parent compound and the metabolite BDB were excreted into sweat. MBDB showed a maximum accumulation at 36 hours post-administration, and the drug appeared in smaller amounts in the patches worn for longer time periods (48 and 72 hours). As in the case of MDMA, reabsorption by the skin was proposed.[130]

4.3.3 Hair

The first experiment on the incorporation of amfetamines in hair was carried out in 1954, and involved identification of amfetamine in the hair of a guinea-pig.[165] Subsequently, the pharmacokinetics of incorporation of amfetamines into the keratin matrix have been extensively investigated in animal models.[151,166-172] In particular, it was shown that incorporation rates of amfetamines from blood to hair were related to substituents on the nitrogen atom of amfetamine, being greater for more lipophilic substituents.[18] The sparse data regarding distribution of amfetamines in human hair after controlled administration have been reported by the same authors.[166,167,169,173] First, it was shown that after metamfetamine ingestion there was a good correlation between duration of drug use and drug distribution.[166] Secondly, controlled administration over 5 days of selegiline 15 mg/day and benzfetamine 30 mg/day, two amfetamine-like substances giving rise to amfetamine or metamfetamine through hepatic biotransformation, showed the usefulness of hair analysis. For selegiline, trace concentrations of this drug, together with desmethylselegiline 0.17−0.19 ng/mg, metamfetamine 1.3−2.5 ng/mg and amfetamine 0.42−0.99 ng/mg were detected in hair collected 3 weeks after the initial dose. The administration of benzfetamine gave rise to 0.14–0.56 ng/mg parent drug, norbenzfetamine 0.29−0.63 ng/mg, metamfetamine 0.1 ng/mg and amfetamine 1.06−1.66 ng/mg.[167,169] Finally, movement of methoxyphenamine along the hair shaft was studied as a model for metamfetamine incorporation. After oral administration of methoxyphenamine 50 mg/day for 7 days and collection of

hair on the first day of the 2 weeks after the first dose, the drug excreted moved along the shaft at a rate of 2.8−3.2 mm/week according to the hair growth, with clearance from the hair matrix cells during 5–8 more days than the periods of drug use.[173]

Furthermore, both classical amfetamines (amfetamine and metamfetamine) and methylenedioxy derivatives have been detected in scalp and body hair of consumers, with maximum concentrations in the range of tens of nanograms per milligram of hair (table IV).[152] Higher hair concentrations of metamfetamine (mean 143.6 ng/mg, range 41.7−250.8 ng/mg) were reached in a fatality after the use of this drug.[170]

4.3.4 Nails

The possibility of detecting drugs of abuse in nails was reported by Suzuki et al.[146] in 1984. Finger and toenail clippings and hair from suspected abusers were analysed for quantification of metamfetamine and amfetamine (table IV). Amfetamine mostly appeared as a metabolite of metamfetamine, being in the concentration ratio of 1 : 30 to metamfetamine in the fingernail. This ratio was higher than that reported in hair (1 : 10), showing that nail matrix tends to accumulate preferentially more lipophilic compounds, even to a greater extent than hair matrix. In the individuals in whom both stimulants could be measured in the fingernails and toenails, concentrations in the toenails were higher, which was related to the slower growth of toenails. Subsequently, Suzuki et al.[155] could quantify metamfetamine and amfetamine in habitual users of metamfetamine and found that the concentration in nails was not correlated with that in hair samples. Recently, methylenedioxy derivatives have been identified in nail specimens of consumers.[156]

As with hair samples, there is a general consensus that determination of amfetamines, like other drugs of abuse, in nails can detect illicit use, although, unlike for nails, the precise moment of drug consumption cannot be established.

4.4 Metabolism

An extensive review on amfetamine metabolism has recently been published by Kraemer and Maurer.[174] As stated by these authors, classical amfetamines as well as metabolic precursors are usually subjected to the main following metabolic reactions: (i) *N*-dealkylation, deamination and oxidation to the corresponding benzoic acid derivatives, which are further conjugated with glycine; and (ii) oxidation at C-4 in the benzene ring with further conjugation of the phenol group with sulphate or glucuronic acid (figure 3 and figure 4). Oxidation at the β-carbon position is a very minor metabolic pathway, giving rise in the case of amfetamine to norephedrine, which can be further oxidised at C-4 in the benzene ring to hydroxynorephedrine.

In vitro data indicate that deamination of amfetamines seems to be catalysed by isozymes belonging to the CYP2C subfamily.[175] Amfetamines methoxylated in the benzene ring or with a methylenedioxy group are in addition *O*-demethylated/demethylenated. If the reaction gives rise to a phenolic group, this is further conjugated with sulphate or glucuronic acid. Amfetamines methoxylated in positions 2 or 3 may additionally be oxidised in position 4. Methylenedioxy derivatives after *O*-demethylenation give rise to a catechol group (which also may happen for 3-methoxyphenamine derivatives oxidised in the fourth position) that is methylated preferentially in position 3 by catecholmethyltransferase and/or conjugated with glucuronide/sulphate (figure 4).

Metabolic reactions involved in the first steps of the disposition of amfetamine derivatives are the most relevant from a toxicological and therapeutic point of view. *In vitro* studies have shown that phenylethylamines (as a class) are to some extent substrates or inhibitors of CYP2D6.[153,176,177] CYP2D6 catalyses the hydroxylation in position 4 of the benzene ring of *N*-alkylated amfetamines (i.e. metamfetamine), the *O*-dealkylation of methoxy amfetamines (i.e. MDMA, MBDB, MDE, PMA) and the *N*-dealkylation of *N*,*N*-dialkylated amfetamines.[178] This isoenzyme is expressed polymorphically in humans, and subjects genotypically classified as poor metabolisers (9% of the Caucasian and 1% of the Asian populations) were postulated to be more susceptible to the acute toxic effects of amfetamine derivatives or to abuse liability.[179] In addition, as CYP2D6 regulates the biotransformation of many therapeutic drugs, it may also be the source of a number of drug interactions

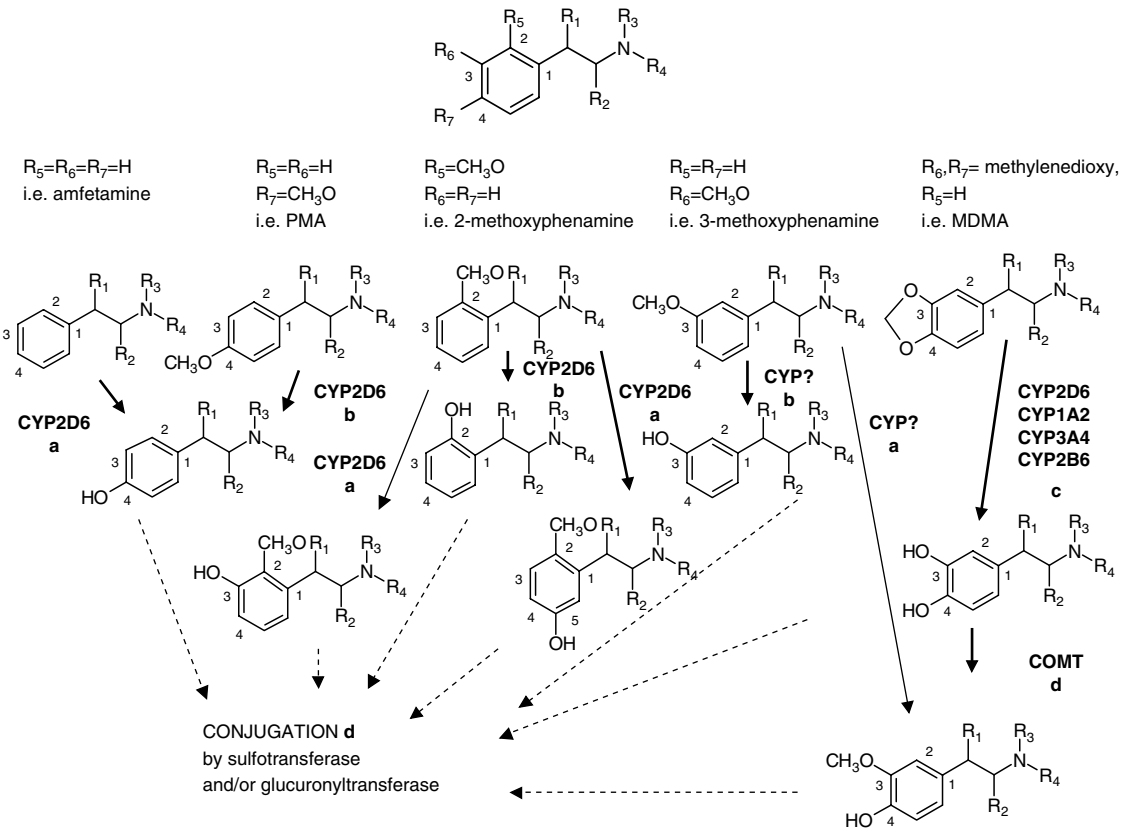


Fig. 3. Metabolic reactions involving the benzene ring moiety of amfetamines, where **a** = ring oxydation; **b** = O-demethylation; **c** = O-demethylenation; and **d** = conjugation. **COMT** = catecholmethyltransferase**; CYP** = cytochrome P450; **MDMA** = 3,4-methylenedioxymetamfetamine; **PMA** = paramethoxyamfetamine.

with the amfetamines. However, clinical data seem not to support fully these expectations.[180] In fact, it has been shown that CYP2D6 overall most probably accounts for less than 60% of MDMA disposition at the metabolic step of *O*-demethylenation; other isozymes involved in this reaction would be CYP1A2 and to a lesser extent CYP2B6 and CYP3A4.[181,182] A CYP-independent mechanism has also been reported for designer drugs.[183] Some authors[183] also reported the *N*-demethylation of MBDB and MDE by CYP3A2/4, which also participates in the demethylenation of many amfetamine-type stimulants.

Furthermore, many amfetamines are administered as racemates. CYP2D6 usually displays a selectivity for one of the enantiomers, which, in the case of MDMA, is the (*S*)-(+)-enantiomer (the more active one). The enantiomer that is not metabolised by CYP2D6, plus the fraction of the active enantiomer not metabolised through this isozyme, undergo metabolic reactions through other CYP isozymes, as already described. Since several isozymes participate in the same reaction, the impact of CYP2D6 genotype on the pharmacology of these drugs is lessened. A further step of complexity is introduced by the enzymatic reaction itself. The *O*-demethylenation reaction of MDMA follows the formation of a metabolite-enzyme complex that inactivates CYP2D6[177,184] and is probably the basis for the nonlinear pharmacokinetics described for MDMA.[118,177,184] The formation of an enzyme complex can also be generalised to other isozymes of CYP and to other phenylisopropylamine derivatives.[185] In practice, methylenedioxy derivatives,

once ingested, have the ability to convert the phenotype of subjects to poor metabolisers for the next dose ingested (within a short period of time), independently of genotype. Even more interestingly, the active enantiomer does not accumulate disproportionately in the body, as the alternative metabolic pathways become more relevant. Most probably, concerns should be more focused on coadministered drugs with a narrow therapeutic index that are substrates of CYP2D6, since these may interact with MDMA. *In vitro* data suggest that individuals homozygous for several functional CYP2D6 allelic variants may have an impaired MDMA disposition capacity. In this respect, the functional allelic variant *CYP2D6*10/*10*, very common among Asians (75%), and variants *CYP2D6*2* and *CYP2D6*17*, more prevalent among African populations, should be considered as increasing susceptibility to toxicity as compared with individuals homozygous for the functional wild-type genotype *CYP2D6*1/*1*.[186,187]

N-Dealkylation of MDMA and MDE is catalysed by CYP2B6,[182] not by CYP2D6 as proposed for metamfetamine.[188] The rate of *N*-dealkylation for methylenedioxy derivatives is nearly of one order of magnitude lower than for *O*-demethylenation, and is characterised by apparently monophasic kinetics. This observation is in contrast with metamfetamine metabolism, where *N*-demethylation is the main metabolic reaction. Both *N*-demethylation and 4-hydroxylation have been proposed to be catalysed by CYP2D6, but at a lower rate than *O*-demethylenation of MDMA. In fact, recoveries in urine of unaltered metamfetamine are much higher than those of methylenedioxy derivatives. There are some discrepancies in the role of CYP2D6 in the *N*-demethylation of metamfetamine because of conflicting *in vitro* results.[188,189] However, the low rates of these metabolic reactions limit their relevance from a clinical point of view.

Selegiline is a good example of the discrepancies between expectations created from *in vitro* data and *in vivo* studies. Selegiline gives rise by *N*,*N*-dealkylation to two metabolites: desmethylselegiline and (*R*)-(−)-metamfetamine. *N*,*N*-Dealkylation of selegiline from *in vitro* data seems to be regulated by CYP2D6,[179] CYP1A2, CYP3A4 and CYP2C19. Poor metabolisers for CYP2C19 showed larger concentrations of desmethylselegiline,[190] whereas poor metabolisers for CYP2D6[191] showed higher concentrations of (*R*)-(−)-metamfetamine.[192] *In vivo* investigations indicate that the two isozymes showing polymorphism in the population, that is CYP2D6 and CYP2C19, seem to contribute marginally to the pharmacokinetics of selegiline. However, the metabolic status of subjects for these two isozymes does not modify the therapeutic effects of selegiline.

Oxidative stress, reactive oxygen species and reactive nitrogen species are known to be involved in neurotoxicity induced by amfetamines.[193-195] In the case of MDMA, it has been postulated that oxidative stress may be mediated by active metabolites resulting from adduct formation with glutathione.[196]

4.5 Excretion

Excretion parameters of the most important amfetamine derivatives, amfetamine, metamfetamine and designer amfetamines, are summarised in table II and table III.

The plasma half-life of amfetamine and related substances is usually highly dependent on the acidity of the urine, since renal excretion is the major elimination route.[137] As amfetamine-derived compounds are weak basic substances, renal excretion is increased by urinary acidification and decreased by urinary alkalinisation.[125] This fact leads to a significant variation in the elimination half-life and, for this reason, amfetamine consumers frequently ingest large amounts of bicarbonate antacids to prolong drug effects.[125] Although the elimination half-life of amfetamine and metamfetamine is not altered by the route of administration, half-life tends to be longer in users with amfetamine dependence as well as in subjects with alkaline urine.[135,137,138] In humans, amfetamine and metamfetamine appear to follow one-compartment pharmacokinetics.[125] The fraction of a dose of amfetamine or metamfetamine administered that is excreted unaltered in the urine is 3−55.5% and 15−55%, respectively, within a range of urinary pH from 5 to 8.[111,197] Furthermore, for metamfetamine, the fraction excreted unchanged in urine decreases with increasing doses and it is not proportional to the dose of the drug absorbed.[111] This observation was attributed to a lower renal elimination rate with increasing doses or, alterna-

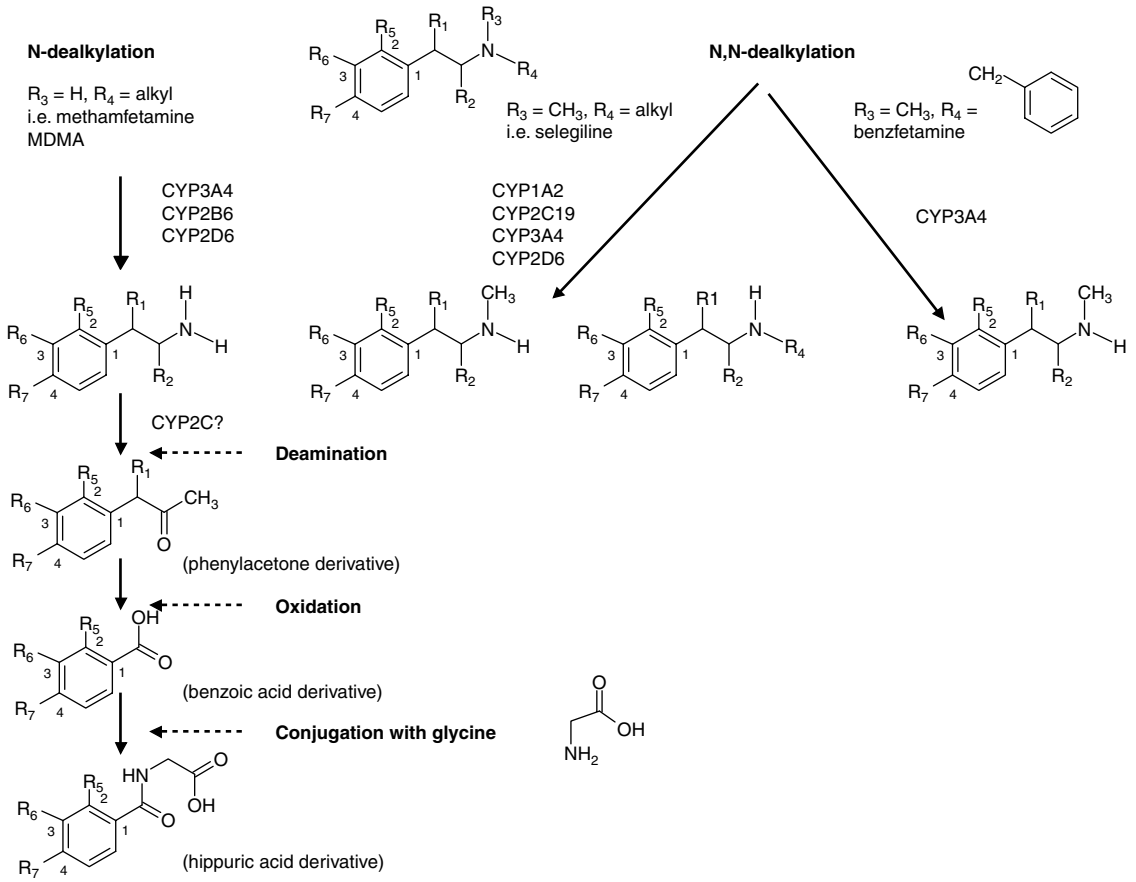


Fig. 4. Metabolic reactions involving the side chain moiety of amfetamines. **CYP** = cytochrome P450; **MDMA** = 3,4-methylenedioxymetamfetamine.

tively, to an increase in the nonrenal elimination rate with increasing doses.

Methylenedioxy derivatives undergo a more extensive metabolism than the prototype compounds, and the amount of unaltered drug excreted in urine is usually smaller. Comparison of renal and total clearance of MDMA shows that about 80% of the drug is cleared metabolically through the liver, and about 20% of the dose is excreted unaltered in urine. Urinary clearance of different MDMA doses appeared to be rather constant. On the contrary, nonrenal clearance was shown to be dose-dependent. The administration of oral MDMA 75mg resulted in a nonrenal clearance of 74.0 ± 71.1 L/h and that of MDMA 125mg in a nonrenal clearance of 38.1 ± 13.3 L/h, suggesting an impairment in MDMA hepatic clearance.[118] After controlled administration of different MDMA doses, the urinary recovery is approximately 60%, independent of the dose given. However, the percentage of unchanged drug accounted for an average of 15%. A similar recovery has been reported for 3,4-dihydroxymetamfetamine (HHMA). Higher recovery rates have been detected for 4-hydroxy-3-methoxymetamfetamine (HMMA), the main metabolite found in urine (>20%), with less than 2% of the dose excreted as MDA.[118,120,154] The elimination half-life of MDMA, in the range of 6–9 hours,[119,154] is lower than those reported for amfetamine and metamfetamine (table III).

Unchanged MBDB was excreted in urine after single oral administration of 100mg.[130] The drug

and its *N*-demethylated metabolite, BDB, were detectable during the first 36 hours, with the ratio of parent drug to metabolite decreasing from 69.1 to 1.3. Enantioselective disposition of MDE, orally administered as racemate, led to a 3-fold increase in the clearance of the (*S*)-(+)-enantiomer and to an almost 2-fold increase in the half-life of the (*R*)-(−)-enantiomer.[61]

Concentrations of unchanged MDA up to 160 mg/L have been reported in fatalities involving direct MDA ingestion, and are indicative of excretion of substantial portions of unchanged drug.[198]

Fatalities involving 4-MTA ingestion showed that unchanged drug is always present in urine specimens.[133,134] The decrease of 4-MTA plasma concentration following a non-fatal intoxication enabled the estimation of its half-life as being approximately 7 hours.[134]

From 49% to 83% of a labelled dose of PMA is excreted in the 24-hour urine, and an average of 15% is eliminated as unchanged drug.[199]

5. Implication of Pharmacokinetic Properties for Application in Toxicology

There are not many amfetamine derivatives left in the pharmaceutical market with well-defined therapeutic applications, which is in contrast to the flourishing market for this type of drugs in the early 1960s in Western countries. On the other hand, during the past decade, these compounds are enjoying the highest popularity in social terms, at least for some young segments of the population. The misuse of these drugs is worrisome in terms of acute and long-term toxicity, and as regulated psychotropic substances their synthesis, trafficking and consumption is controlled. It is in the context of analytical tests performed in humans to control their consumption that the clinical pharmacokinetic data on these drugs are of relevance. There are several situations where the analysis of amfetamines in biological matrices will be of interest. Available clinical pharmacokinetic data have been mainly obtained from blood and urine, the most adequate biological fluids for describing the overall disposition of these drugs from the body. Nevertheless, there is a demand in some circumstances for switching from the classical biological fluids to alternative tissues and/or other fluids representing minor disposition pathways of drugs. More retrospective (in terms of detecting drug consumption) and/or less invasive (in terms of sample procurement) procedures are needed. Nevertheless, the most attractive aspects of alternative matrices for drug control agencies are the possibility of performing on-site testing and the facts that samples need minimal technology for collection (without a need for a medical environment) and that further processing (e.g. whole blood versus plasma, or subfractions) is not required. These alternative matrices and associated analytical technologies facilitate drug testing outside medical settings. However, a consequence of this tendency may be a division between law enforcement authorities and clinical pharmacologists.

Drug law enforcement authorities have already started showing some interest in drug testing in alternative matrices. It is calculated that the Home Office in the UK performs as many as 250 000 saliva tests per year. The renewal of driver's licenses in Italy in subjects known as former drug abusers is dependent on hair testing to verify abstinence.[200] In Germany, approximately 60% of all police forces are routinely using sweat testing at the roadside.[143] In clinical settings, in the context of drug-dependent subjects under treatment or under evaluation for a new treatment, particularly when evaluation of abstinence is indispensable, urine drug testing is progressively being replaced by hair testing.[201] A single hair analysis may reflect past exposure to drugs in the last few months, whereas several urine samples every week have to be analysed to obtain equivalent information. Therefore, testing in alternative matrices is no longer a scientific curiosity but is becoming reality. The procurement of these samples is less invasive than urine drug testing or blood sampling, and is preferred by law enforcement agencies. The genuine interest in these alternative matrices is stimulating industry to develop more and more analytical devices to facilitate testing.

As will be seen from this review, our knowledge of the distribution of drugs, and specifically amfetamines, in these matrices in humans is relatively scarce and heterogeneous, depending on the matrix considered. There are some issues that need to be further investigated in more controlled settings, first to provide better knowledge of the mech-

anisms involved in the diffusion of drugs in these alternative substrates and secondly to establish which are the desired concentrations that should be targeted by analytical devices in each scenario. It is quite apparent that the sensitivity needed to perform saliva testing is not the same as that for hair testing. For those matrices (sweat and saliva) in which concentrations better parallel plasma concentrations, it is very likely that, from a law enforcement perspective, there will be an interest in setting up some kind of threshold concentration. In this forensic context it is relevant to know if a given subject is under the influence of a drug or not. Most of these questions remain open, and the use of these matrices will not be effective until they are answered.

6. Conclusions

From a clinical pharmacokinetic perspective, amfetamine-type substances are quite homogeneous. Oral bioavailability is good, volume of distribution is high (4 L/kg) and plasma binding protein is low (less than 20%). The elimination half-life is greater than 6 hours (range 6–12 hours). Both hepatic and renal clearance contribute to disposition from the body. Hepatic metabolism is extensive in most cases, but a significant percentage of the drug remains unaltered. This observation explains why amfetamine-type stimulants are so ubiquitous in all tissues examined.

In the minor disposition pathways, diffusion is dependent on the physical and chemical properties of the drug and on pH gradients. The pKa of most amfetamine-type substances is around 9, facilitating diffusion across membranes. The relatively high proportion of unaltered drug facilitates its detection in alternative matrices at relatively high concentrations. Experimental data indicate that in most cases concentrations found in alternative matrices are higher than expected from the Henderson-Hasselbach equation. The low protein binding and pKa already suggest good diffusion, but cannot completely explain the experimental data. Most probably, in addition to physicochemical properties, cardiovascular effects and haemodynamic changes induced by the amfetamines, as well as pharmacological effects on some neurotransmission systems, play a role in the diffusion of these drugs. Outside controlled clinical settings, factors influencing disposition will include personal hygiene (dyes and shampooing in hair analysis), exercise habits (reabsorption phenomena in sweat testing) and protocols for sample procurement (induced versus naturally produced saliva).

In summary, the physicochemical and pharmacological properties of amfetamine-type drugs facilitate their diffusion at reasonably high concentrations into biological substrates. Thus, the amfetamines are one of the best suited groups of drugs for drug testing in non-conventional matrices.

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