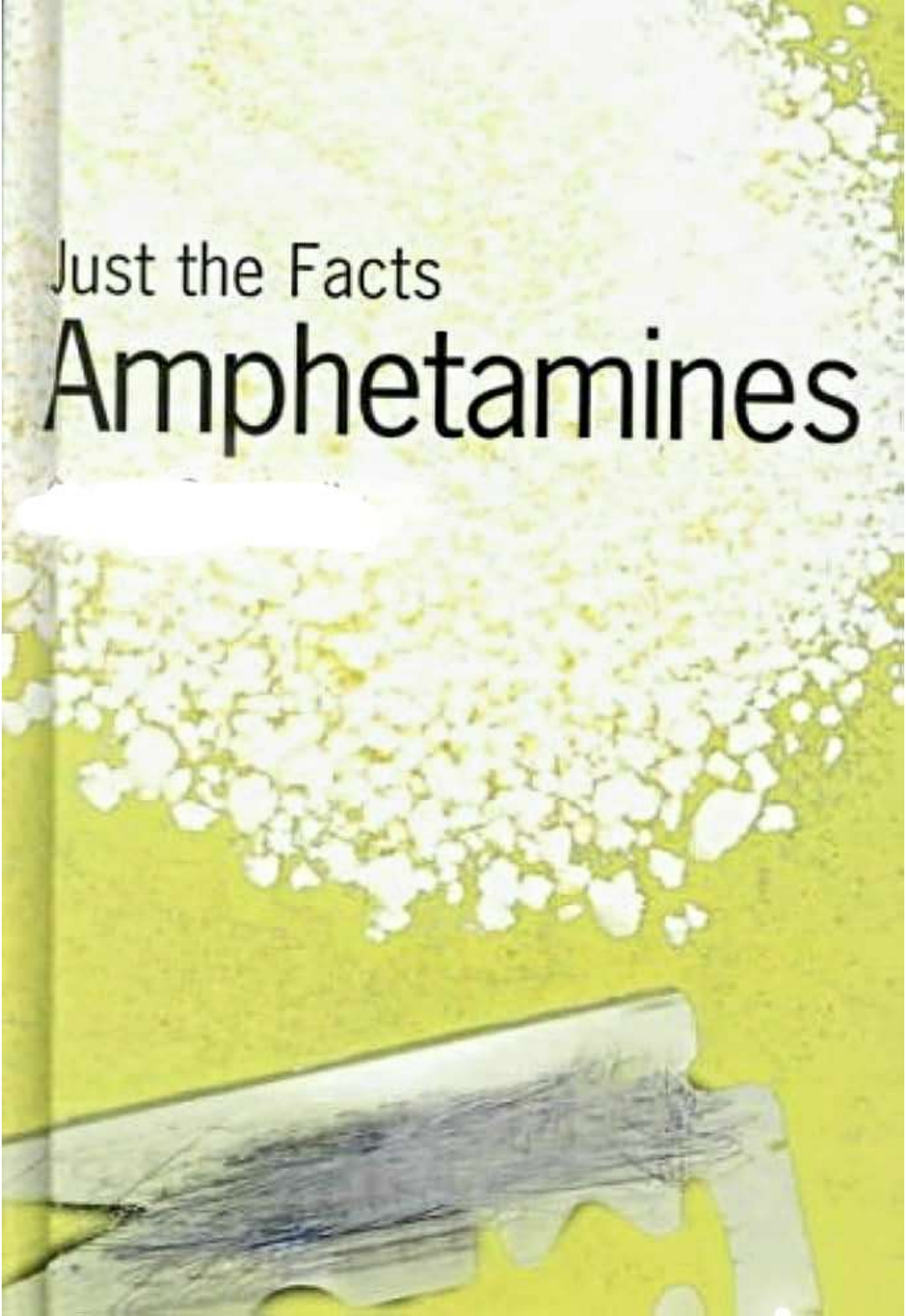
Just the Facts

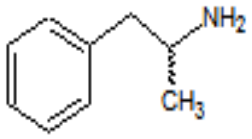
Amphetamines



AMPHETAMINES

1. DRUG PROFILE:-

i. DRUG : Amphetamine
ii. DRUG CLASS :
iii. CHEMICAL NAME:
(+)-alpha-methylphenethylamine Sulfate
iv. BRAND NAMES: Adderall , Vyvanse , and Dexedrine
v. SOURCE: Most species of Acacia carry only a small amount of phenethlamines and tryptamines
vi. ORIGIN OF DRUG: Synthetic
vii. STRUCTURE CHEMICAL:



Amphetamine/Benzedrine
[1-phenylpropan-2-amine]

2 .HISTORY:

- Amphetamine was first synthesized in 1887 by the Romanian Lazăr Edeleanu in Berlin, Germany.
- He named the compound phenylisopropylamine. It was one of a series of compounds related to the plant derivative ephedrine, which had been isolated from Ma-Huang.

- No pharmacological use was found for amphetamine until 1929, when pioneer psycho pharmacologist Gordon Alles resynthesized and tested it on himself, in search of an artificial replacement for ephedrine.
- One of the first attempts at using amphetamines as a scientific study was done by M. H. Nathanson, a Los Angeles physician, in 1935. He studied the subjective effects of amphetamine in 55 hospital workers who were each given 20 mg of Benzedrine.
- The two most commonly reported drug effects were "a sense of well being and a feeling of exhilaration" and "lessened fatigue in reaction to work".
- During World War II amphetamine was extensively used to combat fatigue and increase alertness in soldiers. After decades of reported abuse, the FDA banned Benzedrine inhalers, and limited amphetamines to prescription use in 1965, but non-medical use remained common
- In 1997 and 1998, researchers at Texas A&M University reported finding amphetamine and methamphetamine in the foliage of two Acacia species native to Texas, A. berlandieri and A. rigidula. Previously, both of these compounds had been thought to be human inventions.

3. DOSAGE FORM:

Amphetamines are available in the following dosage forms:

i. ORAL:

a) Tablets

b) Extended-release capsules

c) Extended-release tablets

i. INJECTION:

a) Intravenous
b) Intramuscular

ii. INHALENTS:

a) Snorted
b) Smoked

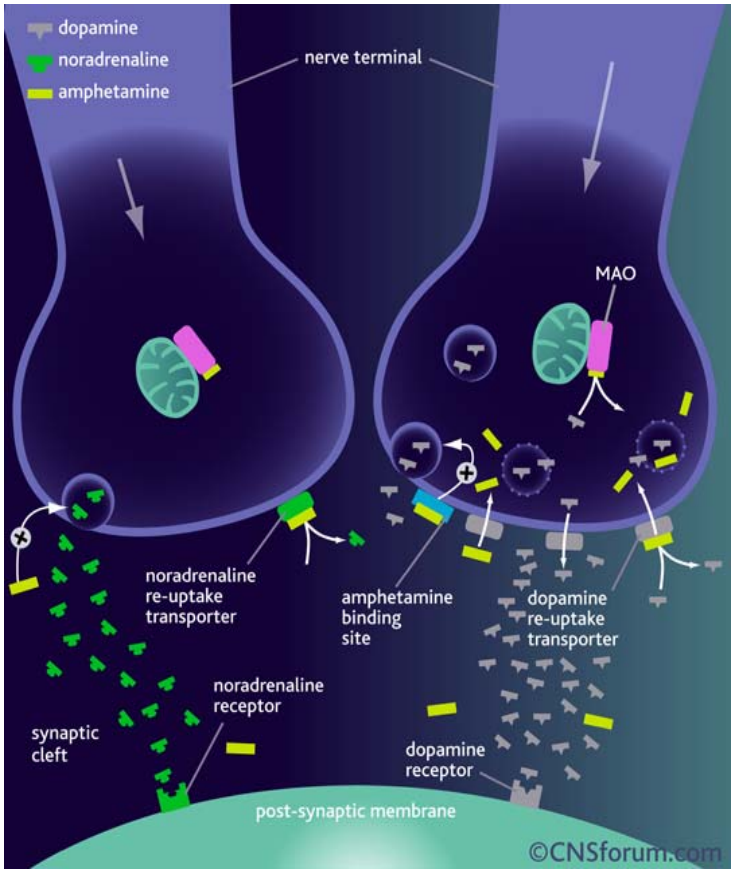
4.PHARMACODYNAMICS:

MECHANISM OF ACTION:

High-dose amphetamine can modify the action of dopamine and nor adrenaline in the brain. At high doses, amphetamine increases the concentration of dopamine in the synaptic cleft in 4 ways:

i. It can bind to the pre-synaptic membrane of dopaminergic neurons and induce the release of dopamine from the nerve terminal.
ii. Amphetamine can interact with dopamine containing synaptic vesicles, releasing free dopamine into the nerve terminal.
iii. Amphetamine can bind to monoamine oxidize in dopaminergic neurons and prevent the degradation of dopamine, leaving free dopamine in the nerve terminal.
iv. Amphetamine can bind to the dopamine re-uptake transporter, causing it to act in reverse and transport free dopamine out of the nerve terminal.

High-dose amphetamine has a similar effect on nor-adrenergic neurones; it can induce the release of nor-adrenaline into the synaptic cleft and inhibit the nor-adrenaline re-uptake transporter.



5. PHARMACOKINETICS:-

i. ABSORPTION:

a) Amphetamine is rapidly absorbed after oral ingestion.
b) Peak plasma levels occur within 1 to 3 hours, vary with the degree of physical activity and the amount of food in the stomach.
c) Absorption is usually complete by 4 to 6 hours.

d) Sustained release preparations are available as resin-bound, rather than soluble, salts. These compounds display reduced peak blood levels compared with standard amphetamine preparations, but total amount absorbed and time to peak levels remain similar.

ii. DISTRIBUTION:

a) Amphetamines are concentrated in the kidney, lungs, cerebrospinal fluid and brain.
b) They are highly lipid solubleand readily cross the blood-brain barrier.
c) Protein binding and volume of distribution varies widely, but the average volume of distribution is 5 L/kg body weight.

iii. METABOLISM:

a) The major metabolic pathway for amphetamine involves the deamination by cytochrome P_{450} to para-hydroxyamphetamine and phenylacetone; this latter compound is subsequently oxidized to benzoic acid and excreted as glucuronide or glycine (hippuric acid) conjugate.
b) Smaller amounts of amphetamine are converted to nor-ephedrine by oxidation. Hydroxylation produces an active metabolite, O-hyroxynorephedrine, which acts as a false neurotransmitter and may account for some as drug effect, especially in chronic users.

iv. ELIMINATION or EXCRETION:

a) Normally 5 to 30% of a therapeutic dose of amphetamine is excreted unchanged in the urine by 24

hours, but the actual amount of urinary excretion and metabolism is highly pH dependent.

6. BIOLOGICAL HALF-LIFE:

a) Under normal conditions, about 30% of amphetamine is excreted unchanged in the urine but this excretion is highly variable and is dependent on urinary pH
b) When the urinary pH is acidic (pH 5.5 to 6.0), elimination is predominantly by urinary excretion with approximately 60% of a dose of amphetamine being excreted unchanged by the kidney within 48 hours.
c) When the urinary pH is alkaline (pH 7.5 to 8.0), elimination is predominantly by deamination (less than 7% excreted unchanged in the urine); the half-life ranging from 16 to 31 hours

7. INDICATIONS:

i. Narcolepsy
ii. Attention Deficit Disorder with Hyperactivity
iii. Exogenous Obesity

8. DRUG INTERACTION:

i. ALCOHOL - may increase serum concentration of amphetamine.

ii. ASCORBIC ACID- lowering urinary pH, may enhance amphetamine excretion

iii. FURAZOLIDONE - amphetamines may induce a hypertensive response in patients taking furazolidone.

iv. LITHIUM CARBONATE - isolated case reports indicate that lithium may inhibit the effects of amphetamine.

v. SODIUM BICARBONATE - large doses of sodium bicarbonate inhibit the elimination of amphetamine, thus increasing the amphetamine effect.

vi. TOBACCO SMOKING - amphetamine appears to induce dose-related increases in cigarette smoking.

vii. ANTIDEPRESSANTS, TRICYCLIC amphetamines may enhance the activity of tricyclic or sympathomimetic agents; d-amphetamine with desipramine or protriptyline and possibly other tricyclics cause striking and sustained increases in the concentration of d- amphetamine in the brain; cardiovascular effects can be potentiated.

viii. ANTIHYPERTENSIVES -amphetamines may antagonize the hypotensive effects of antihypertensives.

ix. MEPERIDINE - amphetamines potentiate the analgesic effect of meperidine.

x. NOREPINEPHRINE - amphetamines enhance the adrenergic effect of norepinephrine.

xi. VERATRUM ALKALOIDS - amphetamines OVERDOSE inhibit the hypotensive effect of veratrum alkaloids.

xii. ETHOSUXIMIDE - amphetamines may delay intestinal absorption of ethosuximide.

9. SIDE EFFECTS:

i. CARDIOVASCULAR: Palpitations, tachycardia, elevation of blood pressure.

ii. CENTRAL NERVOUS SYSTEM: Psychotic episodes at recommended doses (rare), overstimulation, restlessness, dizziness, insomnia, euphoria, dyskinesia, dysphoria, tremor, headaches, exacerbation of motor and phonic tics and Tourette's syndrome.

iii. GASTROINTESTINAL: Dryness of the mouth, unpleasant taste, diarrhea, constipation and other gastrointestinal disturbances. Anorexia and weight loss may occur as undesirable effects when amphetamines are used for other than the anorectic effect.

iv. ALLERGY: Urticaria

v. ENDOCRINE: Impotence, changes in libido.

Adverse effects:

Short term:

- **Dilated Pupils**
- **Increased Blood Pressure**
- **Increased Heart Rate**
- **Decreased Appetite**
- **Dry Mouth**

Long term effects:

Malnutrition **– Because Amphetamines reduce the users appetite, they are less likely to eat properly and because of this are less resistant to infections.**
Chronic Psychosis **– Symptoms include paranoia, delusions, and bizarre behavior. This can be seen as early as 1 week after the user has stopped using.**
Chronic Anxiety/Tension **- To combat this users often turn to alcohol and barbiturates for help. Chronic Anxiety may also lead to violent behavior.**
Brain Damage **– Long term use of Amphetamines can cause damage to the brain, specifically areas that deal with memory and everyday thinking**

Clinical trials:

Phase-1:

Dispensed drugs…… 20-100(Healthy volunteers)
Drug administration……low dose
Dose……..well tolerated
Drug show…….pharmacokinetic & pharmacodynamic
Investigation……..extended to mutidose studies

Phase-2:

Dispensed drugs……..300-500(patients)
Effects of drug……..efficacious
Side effects:

- **Blood pressure**
- **Restlessness**
- **Dizziness**
- **Insomnia**

Dose……..well tolerated
So phase-3……..conducted

Phase-3:

Dispensed drugs……..1000-3000(patients)
Effects of drug……..efficacy, ADR
Adverse reactions:

- **Abdominal Pain (stomachache)**
- **Asthenia (fatigue)**
- **Fever**
- **Infection**

Not show severe adverse drug reactions
Show good actions by……. Orally,IV, Inhalents
Phase-3 approved……ready for marketing

Phase-4 :
Studies during marketing:
Dispensed drugs……..3500(patients)
Effects of drug……..Maximum efficacy,drug interactions
Long term use…… *Malnutrition, Chronic Psychosis,Brain Damage*
Drug Interactions

- ALCOHOL
- ASCORBIC ACID
- FURAZOLIDONE
- LITHIUM CARBONATE

- SODIUM BICARBONATE
- TOBACCO SMOKING
- ANTIDEPRESSANTS
- ANTIHYPERTENSIVES

Marketing data:

The company who launched this brand in Pakistan:

Eon laboratories:

The employees of Eon Labs are dedicated to develop, manufacture and be first to market with a broad range of affordable multi-source pharmaceutical products.

Eon Labs, Inc. announced today that they received final approval for the generic alternative for the ADHD treatment, Adderall(R),

(a)The brand had sales of nearly $500 million in 2001,

The marketing price of adderall are

Adderall (Branded Version) - #60 tabs

- 5 mg tabs - $86.99
- 10 mg tabs - $77.99
- 20 mg tabs - $77.99

Research paper:

Methamphetamine and Amphetamine Pharmacokinetics in Oral Fluid and Plasma after Controlled Oral Methamphetamine Administration to Human Volunteers

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Abstract:

Background: Meth**amphetamine** (METH) and **amphetamine** (AMP) concentrations in 200 plasma and 590 oral fluid specimens were used to evaluate METH **pharmacokinetic**s and pharmacodynamics after oral administration **of** sustained-release METH.

Methods: Eight participants received four oral 10-mg *S*-(+)-METH hydrochloride sustained-release tablets within 7 days. Three weeks later, five participants received four oral 20-mg doses. Blood samples were collected for up to 24 h and oral fluid for up to 72 h after drug administration.

Results: After the first oral dose, initial plasma METH detection was within 0.25–2 h; c_{max} was 14.5–33.8 µg/L (10 mg) and 26.2–44.3 µg/L (20 mg) within 2–12 h. In oral fluid, METH was detected as early as 0.08–2 h; c_{max}

was 24.7–312.2 μg/L (10 mg) and 75.3–321.7 μg/L (20 mg) and occurred at 2–12 h. The median oral fluid-plasma METH concentration ratio was 2.0 across 24 h and was highly variable. Neutral cotton swab collection yielded significantly higher METH and AMP concentrations than citric acid candy-stimulated expectoration. Mean (SD) areas under the curve for AMP were 21% ± 25% and 24% ± 11% **of** those observed for METH in plasma and oral fluid, respectively. After a single low or high dose, plasma METH was >2.5 μg/L for up to 24 h in 9 **of** 12 individuals (mean, 7.3 ± 5.5 μg/L at 24 h); in oral fluid the detection window was at least 24 h (mean, 18.8 ± 18.0 μg/L at 24 h). The plasma and oral fluid 24-h METH detection rates were 54% and 60%, respectively. After four administrations, METH was measurable for 36–72 h (mean, 58.3 ± 14.5 h).

Conclusions: Perceived advantages **of** oral fluid for verifying METH exposure compared with urine include simpler specimen collection and reduced potential for adulteration, but urine **of**fers higher analyte concentrations and a greater window **of** detection.

Introduction :

Meth**amphetamine** (METH),[1] a potent, highly addictive stimulant, was synthesized for therapeutic use in the early 1900s (1). METH abuse is a serious problem in the US, Mexico, South America, the Middle East, the Arabian peninsula, Asia, and Australia (2)(3)(4).

METH, like other **amphetamine**s, is a sympathomimetic drug. When administered intravenously, small doses have prominent central stimulant effects (5)(6)(7). In humans, METH is readily absorbed from the gastrointestinal tract after oral consumption (8). METH is almost entirely

(90%) eliminated in urine (9). In urine **of** pH 6–8, 22% **of** a METH dose is excreted as unchanged drug, 15% as *p*-hydroxymeth**amphetamine** (pOH-METH), 4–7% as **amphetamine** (AMP), and 1% as *p*-hydroxy**amphetamine** (pOH-AMP) (8)(9). **Of** quantitatively minor importance are phenylacetone and the hydroxylation products **of** AMP and pOH-AMP, i.e., norephedrine and *p*-hydroxynorephedrine (10). During phase two biotransformation, the majority **of** pOH-METH and pOH-AMP is conjugated with glucuronic acid (9). The renal excretion **of** METH and AMP is enhanced by urinary acidification, producing shortened plasma elimination half-lives and increased total clearance (11).

Oral fluid is an alternative biological matrix to urine and plasma for drugs-**of**-abuse testing (12). Interest has increased because **of** the ease, noninvasiveness, and safety **of** specimen collection. Today, its usefulness as an aid in clinical diagnosis and for therapeutic drug monitoring is established (12). Indeed, oral fluid testing has been successfully used as an alternative to blood testing in **pharmacokinetic** and pharmacotoxicologic studies; in many cases, drug in oral fluid represents the physiologically active fraction (13)(14).

For therapeutic drug monitoring (TDM) purposes, the usefulness **of** oral fluid as a test matrix is dependent on consistent oral fluid-plasma (S-P) ratios (15). For oral fluid concentrations to accurately predict plasma concentrations, the S-P ratio must be independent **of** drug concentration and consistent within and across individuals. In practice, oral fluid is used only to monitor a select array **of** drugs because **of** large intra- and intersubject variability in S-P ratios. This variability can be explained by a multiplicity **of** factors that control drug disposition from plasma. In theory, a drug circulating in plasma must cross the capillary membrane, the basal membrane, and glandular epithelial cells **of** the salivary gland before it can pass into oral fluid. The mechanisms involved in these distribution processes remain largely unknown for many substances, including METH and AMP. One or more **of** the following transport processes could be involved in the transport **of** METH and AMP from plasma to oral fluid: *(a)* passive diffusion, *(b)* facilitated diffusion (a carrier-mediated, non-energy-consuming process), or *(c)* a carrier-mediated active transport mechanism. As opposed to TDM, the use **of** oral fluid for drug detection is not dependent on predicting plasma concentrations; rather it is used to detect drug use. Therefore, S-P ratios are less important.

The **pharmacokinetic**s **of** METH and AMP in plasma and oral fluid in humans after controlled METH administration have been described in only two reports, both from Cook and coworkers (16)(17): one after oral METH administration (16), and the other after METH vapor inhalation (17). Other reports providing information about METH **pharmacokinetic**s do not include oral fluid data or the effect **of** different oral fluid collection methods on METH drug concentrations. Shappell et al. (18) correlated plasma METH concentrations with chronopharmacodynamic data. Perez-Reyes and coworkers (19)(20) focused on the pharmacodynamic data from the above-mentioned clinical studies conducted by Cook and coworkers (16)(17). Driscoll et al.(21) determined the mean blood METH concentrations **of** 10

females after oral METH intake, and Suzuki et al. (22) analyzed METH and AMP in various biological matrices, including 20 oral fluid specimens collected from drug abusers.

The aims **of** this study were to describe the **pharmacokinetics of** METH and AMP in human oral fluid and plasma after controlled oral METH administration, to determine whether oral fluid concentrations can be used to predict plasma concentrations, and to compare oral fluid collection methods. In addition, these METH and AMP concentration data will strengthen the interpretation **of** oral fluid test results and will aid in establishing the detection window **of** METH in oral fluid. Concurrent pharmacodynamic effects **of** oral METH administration were also investigated.

Materials and Methods:

participants and study design
The study was approved by the National Institute on Drug Abuse (NIDA) Institutional Review Board. Throughout the study, participants resided in the Intramural Research Program's secure clinical ward at NIDA. All participants provided written informed consent and were paid for their participation.

Before admission, each participant underwent thorough medical, physical, and psychologic evaluations, including a history of past and recent drug use. Participants resided on a closed clinical ward for 2 weeks before METH administration to permit elimination of previously self-administered drugs.

Participants received four daily 10-mg (low) oral *S*-(+)-methamphetamine · HCl doses within 7 days. After a 3-week interval, five of eight participants received four daily 20-mg (high) oral doses of *S*-(+)-methamphetamine · HCl. Two participants were disqualified from the study for medical reasons. A third participant chose to discontinue the study after the fourth dose. Participants received a single gelatin capsule containing one or two Desoxyn® Gradumet® 10-mg sustained release tablets (Abbott Laboratories) with lactose (Amend Drug & Chemical Co., Inc.) as the filler. For placebo treatments, the capsule contained lactose only.

All drug administrations were conducted under subject-blind conditions. The Gradumet formulation was developed to sustain a slow release of drug after oral administration. Dissolution tests of the preparation showed that 20–40% of the drug is released after 30 min, 35–55% after 1 h, and 60–80% after 4 h. Desoxyn Gradumet 5 mg tablets are currently available.

METH is commonly abused via smoking, insufflation, injection, and less commonly, via the oral route (23). In our controlled administration study in human volunteers, a sustained-release oral METH formulation was used because of health and safety concerns for study participants.

specimen collection
Participants consumed a light breakfast in the morning and did not smoke or eat for 1 h before the session. Oral fluid was collected 15 min before; 5, 10, 15, and 30 min; and 1, 2, 4, 8, 11.5, and 24 h after drug administration. Three oral fluid collection methods were used. During the first two sessions, a citric acid sourball candy with 90.3 ± 3.3 mg citric acid (Brach's Confections Inc.) stimulated oral fluid production. Participants expectorated

5 mL of oral fluid into a 50-mL screw cap polypropylene collection tube. During session three, oral fluid was collected by placing a cotton swab treated with 20 mg of citric acid (Salivette® cotton swab citric acid preparation; Sarstedt International) in the volunteer's mouth. During the fourth session, cotton swabs without citric acid (Salivette cotton swab without preparation; Sarstedt) were used for oral fluid collection. The liquid was extracted from the swab by centrifugation. Samples were transferred into polypropylene tubes and stored at -20 °C until further analysis.

Venous blood was drawn from the forearm through a heparin lock and transferred to a sodium heparin-containing Vacutainer® (Becton Dickinson) to which 87 µL of a saturated sodium fluoride solution and 87 µL of a solution of 100 mL/L glacial acetic acid in water were added. After centrifugation, plasma was isolated, and specimens were stored in a polypropylene tube at -20 °C until analysis. During the first session of both the low- and high-dosage regimens, blood samples were collected simultaneously with oral fluid for up to 24 h. During all other sessions, a single blood sample was collected 24 h after drug administration. For the well being of participants, limitations were imposed on the total amount of blood that could be collected during the study.

collection of pharmacodynamic data
Physiologic data were collected during the first low session, first high session, and a placebo session with a Datascope® Passport® Model EL physiologic monitor (Datascope Corporation). Pupillometry was assessed with use of an adapted Polaroid camera. Within each session, systolic and diastolic blood pressure, heart rate, respiration rate, oxygen saturation, skin and core temperature, and pupil diameter were recorded at baseline (15 min before dosing); 5, 10, 15, 30, 45, 60, 75, 90, and 105 min; and 2, 4, 8, and 11.5 h after drug administration. No physiologic data were collected for volunteer V.

specimen analysis
Samples were analyzed for METH and AMP by solid-phase extraction and gas chromatography–mass spectrometry procedures similar to those described by Huestis et al. (24) for the simultaneous determination of opiates, cocaine, and their metabolites. The method of analysis was developed further to simultaneously determine METH, cocaine, codeine, and their metabolites so that a single analytical method could be used for the analysis of samples collected in multiple drug administration studies.

To 1 mL of specimen were added 100 µL of a 1 µg/L aqueous solution of internal standards (d_{11}-METH and d_{10}-AMP) and 3 mL of 2.0 mol/L sodium acetate buffer (pH 4.0) in a 4-mL fritted reservoir (UCT Inc.). Samples were allowed to stand for 10 min and were centrifuged at 3000*g* for 5 min. The supernatant was collected in 16 x 100 mm glass test tubes and decanted onto CSDAU Clean Screen Extraction columns (UCT Inc.) preconditioned with 1 mL of a mixture of methylene chloride, 2-propanol, and 14.5 mol/L ammonium hydroxide (80:20:2 by volume) followed by 3 mL of methanol, 3 mL of deionized water (twice), and 1.5 mL of 2 mol/L sodium acetate buffer (pH 4). Vacuum was applied, and columns were washed sequentially with two 1-mL aliquots of water, 1.5 mL of 0.1 mol/L HCl, and two 1-mL aliquots of methanol.

After the solid-phase extraction columns were dried, analytes were eluted four times with 1 mL of a mixture of methylene chloride, isopropanol, and 14.5 mol/L ammonium hydroxide (80:20:2 by volume). The combined eluates were collected in conical glass centrifuge tubes with 20 µL of *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide containing 10 g/L *tert*-butyldimethylchlorosilane (MTBSTFA +1% TBDMCS; Pierce) to reduce METH and AMP volatility. Samples were then evaporated under a

continuous nitrogen stream in a water bath at 40 °C until dry. A 500-µL aliquot of acetonitrile was added, and tubes were vortex-mixed to recover drug from centrifuge tube walls. After samples were evaporated to dryness, 20 µL of acetonitrile was added and centrifuge tubes were capped, vortex-mixed, and centrifuged at 3000*g* for 5 min. Samples were transferred to autosampler vials, and 20 µL of MTBSTFA was added. Vials were loosely capped and placed in a heat block at 80 °C for 15–20 min. We then added 20 µL of *N,O*-bis(trimethylsilyl)trifluoroacetimide containing 10 g/L trimethylchlorosilane (BSTFA +1% TMCS; Pierce), crimp-capped the vials, and heated them at 80 °C for 45 min.

Analyses were performed on a Hewlett-Packard Model 6890 gas chromatograph equipped with a Hewlett-Packard Model 6890 autosampler and a 12 m x 0.2 mm (i.d.) HP-1 capillary column (0.33 µm film thickness) or a 15 m x 0.2 mm (i.d.) Phenomenex® ZB1 capillary column (0.10 µm film thickness) interfaced with a Hewlett-Packard Model 5973A electron impact mass spectrometer. Helium was used as carrier gas (flow rate, 1 mL/min) for 1-µL splitless sample injections. The initial oven temperature was 70 °C with a 1-min hold followed by ramps at 35 °C/min to 200 °C, 25 °C/min to 250 °C, and 21 °C/min to 325 °C with a 1-min final hold. Total run time was

11 min. Specimens were analyzed in selected-ion monitoring mode for the following ions (where q is the quantitative ion): d_{11}-METH [trimethylsilyl (TMS) derivative], *m/z* 136 (q), 96, and 217; METH (TMS derivative), *m/z* 130 (q), 91, and 206; d_{10}-AMP [*tert*-butyldimethylsilyl (TBDMS) derivative], *m/z* 162 (q), 202, and 244; AMP (TBDMS derivative), *m/z* 158 (q), 192, and 234. Samples were accepted for quantification if both target ion ratios were within ± 20% of the values computed for the 10 µg/L calibrator (100 µg/L internal standard). In addition, chromatography had to be acceptable for all ions, and retention times had to be within ± 2%.

A total of 590 oral fluid and 200 plasma specimens were analyzed. METH and AMP peak areas were integrated using the HP ChemStation Software® (Rev. C.00.00), and the ratio of the area of the calibrator and its internal standard were used for calculations. For each sample batch, two calibration curves, i.e., 2.5–50 and 50–500 µg/L, were generated to obtain adequate sensitivity and linearity (r >0.993). The limits of detection (LOD) and quantification (LOQ) were 2.5 µg/L for both analytes. LODs and LOQs were determined by analyzing serially diluted blank oral fluid specimens to which METH and AMP had been added. The LOD of the analyte was based

on its correct retention time, a signal-to-noise ratio for all ions of 3:1, and qualifier ratios within ± 20% of those observed with a 10 µg/L calibrator. In addition to the LOD criteria, the LOQ required quantification within 20% of the target concentration for three of four replicates. Duplicate control samples at 12.5, 25 and 250 µg/L were included in each batch and were required to be within 20% of their theoretical value. Between-run imprecision, as measured with duplicate control samples at 12.5, 25, and 250 µg/L (n = 10 sets of duplicates), was 12%, 10%, and 5.3% for oral fluid METH and 7.5%, 7.8%, and 4.2% for oral fluid AMP, respectively; the between-run imprecision for plasma was 4.3%, 3.5%, and 1.4% for METH and 1.7%, 2.8%, and 1.9% for AMP for the same set of controls, respectively (n = 7 sets of duplicates).

PH measurements of oral fluid specimens
The pH of oral fluid specimens was measured using pH indicator sticks (JT Baker). Small aliquots of oral fluid samples were spotted on the pH indicator sticks, and the color of the sticks was compared with a color scale.

The pH resolution was 0.4 pH units.

pharmacokinetics and statistical analysis
The pharmacokinetics of METH and AMP were evaluated by use of WinNonlin® (Pharsight Corporation). Noncompartmental analysis included determination of the following parameters: t_{lag}, corresponding to the time before the first measurable (non-zero) concentration; t_{max}, the time to maximum observed concentration; c_{max}, the concentration corresponding to t_{max}; $t_{1/2}$, estimated via linear regression of time vs the log terminal end portion of the curve; and $AUC_{0–24\ h}$, the area under the curve from the time of dosing to the time of the last observation (24 h). Volume of distribution (V_d) values were expressed in L/kg of body weight and were computed based on the terminal elimination phase; Cl represents the total body clearance for extravascular administration. AUCs were calculated by the linear trapezoidal rule. Data are reported as the mean ± SD unless otherwise defined. Plasma data from individual participants were evaluated with a one-compartment model with first-order input and output and computation of absorption (K_{01}) and elimination constants (K_{10}). The evaluation of best fit was based on examination of the CVs for the parameter estimates and on the Akaike criterion (25).

Plasma METH concentrations after the first low and high dose were compared using a paired Student *t*-test of their respective log-normalized AUC values. To investigate the effect of dose and collection device (i.e., the fixed effects) on oral fluid METH concentrations while taking into account random subject effects, we used SAS® software incorporating a repeated-measures mixed-effects ANOVA. Post hoc analysis of these data with the Scheffé pairwise test enabled us to verify sign and significance of the identified effects.

Similarly, to investigate the effects of subject (random variable), dose, and collection device (fixed effects) on oral fluid pH, we used SAS software incorporating the General Linear Model procedure for mixed-model ANOVA. Post hoc analysis of the data with the Scheffé pairwise test gave sign and significance of the identified effects.

For the analysis of physiologic data, the AUC values of the time–effect curves were calculated. The effect of METH dose on the physiologic response was investigated by use of a one-way ANOVA. Post hoc analysis was performed with the Bonferroni test, allowing for pairwise comparisons of low dose, high dose, and placebo physiologic parameter AUCs.

All data were screened for outliers by use of box plots. No outliers were detected except for S-P ratios, which were subsequently subjected to nonparametric testing. Thus, all data were included in all calculations. The

level was set at 0.05 for all analyses. Effects were considered statistically significant at $P < 0.05$.

Results:

participant data

Four males and four females participated in the study. Their mean age was 35.3 ± 4.2 years (range, 26.3–39.8 years); their mean height was 171.5 ± 9.8 cm (range, 149.9–177.8 cm), and their mean weight was 72.0 ± 17.6 kg (range, 54.7–103.2 kg). All eight participants received four low doses, and volunteers S, W, Y, AA, and BB (n = 5) received four high doses. No plasma data were available for volunteer Z after the first low dose because **of** blood collection difficulties.

meth and amp pharmacokinetics in plasma and oral fluid

Results for plasma and oral fluid METH after the first low and high METH

doses are summarized in Table 1 and are presented graphically in Fig. 1. METH was initially detected in plasma after both doses within 0.25–2.0 h (n = 12). After the first low dose (n = 7), maximum METH concentrations averaged 20.2 ± 6.4 μg/L (range, 14–34 μg/L) within 2–8 h. After the first high dose (n = 5), peak plasma METH concentrations averaged 32.4 ± 7.7 μg/L (range, 26–44 μg/L) and occurred at 2–12 h post dose. The 24-h AUCs for plasma METH after the first low and high dose were significantly dose dependent and were, on average, 2.1 ± 0.7 (range, 1.2–3.1) times higher after the high dose than after the low dose (P = 0.009).