

# TOXICOLOGY REPORTER

## DRUGS

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*Specializing in Toxicology*

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# TOXICOLOGY REPORTER

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## DRUG-RELATED TOPICS

### Fate of Drugs

#### Consumption-Absorption-Distribution-Elimination and Case-illustrations

#### Therapeutic Drug Monitoring

#### Premortem Drug Testing

#### Hospital Drug Testing Probationary Drug Testing GC and GC-MS Drug Testing

#### Postmortem Drug Testing

#### Discovery – Review - Retesting

#### Effects of Drugs

#### Drugs and Accident Drugs and Behavior

#### Death Cases

#### Terminology

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## The Fate of Drugs

### Illustration: Cocaine and Opiates

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#### Drug use

Drug use can be described by terms that include the following:

- Acute (recent) use
- Chronic (longer-term) use
- Therapeutic (medicinal) use
- Experimental use
- Social-recreational use
- Situational use
- Compulsive use

#### Route of entry

Routes of drug use include oral consumption, inhalation, intravenous injection, nasal insufflation, and topical administration. The route of drug use will affect

bioavailability, metabolism, distribution, site-specific drug concentrations, and the pharmacodynamic response (i.e. the nature, extent, and time-course of the response following drug use).

#### Absorption

The absorption of most drugs is a passive process: The drug moves from a location of higher concentration to a location of lower concentration. However, the rate and time-course of absorption are affected by a number of factors. For example: With pills and capsules, the rate-controlling factors affecting drug absorption include the dissolution rate, gastric emptying time, and chemical features related to the ionization of the drug. If an oral drug overdose initially results in physiologic effects that decrease gastrointestinal motility and/or gastric emptying time, it can take a long time to reach the peak or highest drug concentration in blood.

Many drugs are absorbed more slowly while in the stomach compared to the small intestine. In such cases, the speed with which the stomach is emptied into the small intestine (i.e. the gastric emptying time) affects the overall rate of absorption. In addition to some drug-related effects, the consumption of food can slow (i.e. delay) gastric emptying, increase the time required to reach the peak or highest blood drug concentration (BDC), and reduce the peak BDC. Under some circumstances, it can take more than two hours after last consumption to reach the peak or highest BDC.

*T-1/2 absorption* refers to the time required for one-half of the dose to be absorbed.

*Tmax* usually refers to the time it takes for the BDC to reach its peak or highest level following administration.

*Cmax* usually refers to the highest BDC following a dose of the drug.

#### Distribution, drug ratio, and equilibrium

Distribution refers to the processes involved in the movement of drugs within and between body tissues and fluids (i.e. physiologic compartments). A drug ratio for two or more specimens (e.g. BDC compared to tissue drug concentration) refers to the relative concentration at a specific time. When the BDC is rising during an absorptive phase, states of disequilibria occur ... drug moves at the site of absorption, drug is distributed to and between body tissues and fluids, and both absolute and relative drug concentrations change. As a general rule, drug equilibrium is more closely approached during a steady-state period or during a post-absorptive period when the BDC is falling.

Expected or observed drug concentration ratios have been reported for many classes of drugs (including barbiturates, amphetamines, benzodiazepines, tricyclic antidepressants, and opiates) and for tissues and fluids that include blood, blood serum, blood plasma, cerebrospinal fluid, brain, lung, and liver.

Theoretical or expected equilibrium ratios are controlled by specimen:specimen differences such as the content of water or fat or protein, while the rates with which these ratios respond to changes in BDCs during the absorptive and post-absorptive state are controlled by processes that include blood flow between and within specific organ(s) or physiologic compartment(s).

Examples of post-absorptive physiological processes that are related to pharmacodynamic response include the following:

Arterial and venous blood flow moving drug to and between body tissues and fluids

Diffusion of drug across biological blood:tissue barriers affecting the amount (concentration) of drug at the site of action or in other body tissues or fluids

Differential solubility or binding of drugs in body tissues and fluids resulting in differential drug concentration ratios

Binding to biological components of blood such as blood cells and/or blood proteins which affects both the amount of drug that is available to enter tissues and the relationship between the concentration of drug in whole blood or blood serum and the expected effect of the drug

Test results indicating an apparent blood:fluid or blood:tissue concentration ratio can sometimes provide information regarding the route of administration and, in drug-related fatalities, the time between last drug use and death. Two examples follow.

Abnormally high urine:blood drug and drug metabolite ratios would usually suggest a post-absorptive state.

An abnormally high liver:BDC ratio following a fatal oral drug overdose would suggest that death occurred shortly after the drug was last consumed ... during or shortly after the absorptive phase.

When considering a drug concentration ratio, it is important to consider any evidence of a grossly altered physiologic condition (e.g. renal or hepatic failure) that could substantially affect drug distribution or elimination.

### Elimination

Elimination refers to the processes that directly result in a reduction of drug concentration:

Metabolism due to enzyme-controlled processes resulting in the formation of drug metabolites that are usually less biologically active than the parent drug and more readily excreted from the body

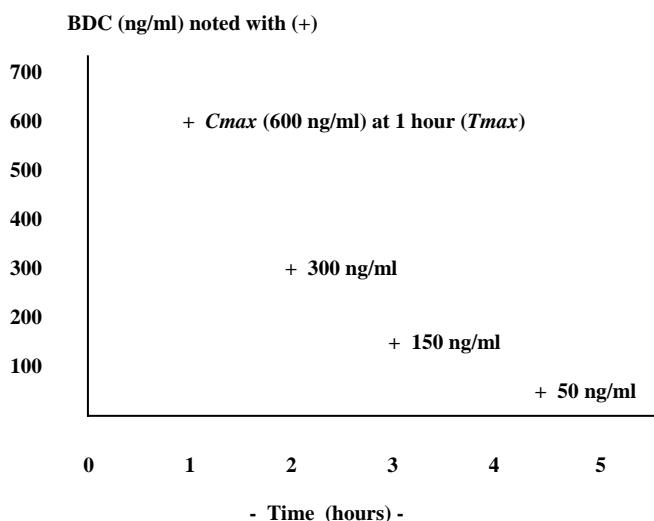
Chemical decomposition that does not involve an enzyme but which usually results in the formation of products that exhibit less biological activity and an increased rate of clearance or excretion

Excretion in biological products including urine or extraction by artificial means such as dialysis

Studies have shown that the biological rate of elimination is affected by variables that include route of drug administration, drug concentration, renal function, and liver function.

### A simulation of drug absorption, distribution, and elimination

The simulation reflects a subject's BDC at  $T_{max}$  (one hour post-consumption) and at specific post-absorptive times consistent with an elimination half-life ( $T-1/2$ ) of one hour. *In this simulation, the incremental decrease in drug concentration is not constant ... it is related to the drug concentration ... it reflects a first-order process.*



When (0) is the time the drug was taken by mouth, (1) is the time of the highest BDC, and BDCs at times between (2-5) reflect an elimination half-life of one hour during the post-absorptive period. *Assuming that the elimination half-life remained constant, the extrapolated BDC would be 20 ng/ml at about six hours, 10 ng/ml at about seven hours, and 5 ng/ml at about eight hours. For this simulation, how many hours post-consumption are required for the BDC to fall to a level that is one-tenth of the  $C_{max}$ ? The answer: About four hours.*

### Effects

Studies relating to pharmacodynamics and the assessment of the risk of adverse drug-related effects or related consequences have included the following topics:

Acute (shorter-term) v. residual (longer-term) effects

Dose:effect and concentration:effect relationships

For example: What is the relationship between BDC and the occurrence or severity of an adverse effect including the risk of accident or death?

Physical and behavioral effects and associated risks

For example: Did the consumption of a drug substantially contribute to his decision to commit suicide?

## Causation v. contribution v. coincidental factors

For example: Was the pedestrian-MVA related to drug use? How? To what extent? What was the risk? What is the degree of certainty?

Degree of certainty or predictability or likelihood

For example: What percent of adults would exhibit one or more visible or obvious indicia of intoxication at a specified BDC?

### An illustration ... Cocaine:

Cocaine is abused by routes of administration that include nasal insufflation or *snorting* of cocaine *powder*, smoking of *crack* or *free-base* cocaine, or injection of a solution of cocaine *powder*. Consuming the same net weights of cocaine by means of injection (1), nasal insufflation (2), and free-basing (3), would likely result in the absorption of weights of drug in the order of  $1 > 2 > 3$ , but the acute CNS effects would likely be  $3 > 2 > 1$  or  $3 > 1 > 2$ . *Note: “>” mean “greater than”.*

Example: The consumption of cocaine by mouth results in chemical decomposition in the gastrointestinal tract and the delayed absorption of the available cocaine. These processes as well as substantial hepatic extraction leading to a much higher post-absorptive concentration of cocaine in liver tissue compared to brain tissue result in reduced CNS effects when cocaine is taken by mouth.

Example: The T-1/2 for the absorption of cocaine powder consumed by nasal insufflation is subject to several variables. An example simulation follows:

#### Nasal Insufflation of Cocaine

Time	Observation	Rate (fraction/min.)
6:00 p.m.	50 mg “snorted”	
6:15	30 mg absorbed	0.60/15 min.
6:30	10 mg absorbed	0.80/30 min.
6:45	5 mg absorbed	0.90/45 min.
7:00	3 mg absorbed	0.96/60 min.
7:15	1 mg absorbed	0.98/75 min.

*In this simulation, the rate of absorption is not constant (i.e. zero-order) ... the rate of absorption is closer to a first-order process reflecting the amount of cocaine not yet absorbed (i.e. the available drug).*

Example: Smoking *crack* cocaine initially results in high drug concentrations in brain tissue leading to the desired *rush* of physical and behavioral effects, followed by drug redistribution and elimination resulting in reoccurring cravings.

Example: Intravenous injection results in very high initial BDCs followed by an initial distribution phase and then an on-going elimination phase. An illustration follows:

## Intravenous Injection of Cocaine

### Illustration: Cocaine Concentration Ratios

Time	Liver (L)	Blood (B)	B/L Ratio
6:00 p.m.	00 mg/Kg	00 mg/L	Injection
6:05	2	70	R = 35
6:10	8	20	R = 2.5
6:15	10	10	R = 1
6:20	12	6	R = 0.5
7:20	7	3	R = 0.44

Example: The oral consumption of cocaine results in intestinal absorption followed by hepatic-extraction leading to high concentrations in liver tissue. On-going absorption-extraction-redistribution-elimination leads to changes in drug concentrations. The following simulation compares the concentration of cocaine in liver and blood following the oral ingestion of part of an “8-ball” of cocaine.

### Oral Consumption of Cocaine

#### Illustration: Cocaine Concentration Ratios

Time	Liver (L)	Blood (B)	B/L Ratio
6:00 p.m.	00 mg/Kg	00 mg/L	Ingestion
6:30	22	01	R = .04
7:00	88	06	R = .07
7:30	99	09	R = .09
8:00	85	10	R = .12
9:00	50	07	R = .14

In some cases, the calculation of blood:tissue drug concentration ratios (Rs) can assist in determining the time between drug overdose and death or the route of drug administration.

Example: If you have a reliable test result, you can sometimes extrapolate the approximate blood cocaine concentration at times prior to specimen collection. The following illustration assumes that the first-order rate of elimination is about one hour.

#### The Apparent Rate of Elimination of Cocaine

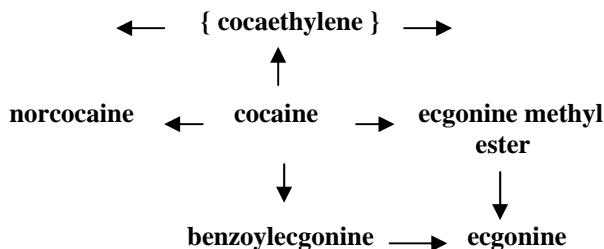
Hour	Cocaine Concentration
5 p.m.	Free-basing ends
6	1.6 mg/L
7	0.8
8	0.4
9	0.2

*In this simulation, the blood cocaine concentration falls, every hour, to one-half of its prior concentration.*

*Question: What would be the extrapolated cocaine concentration at 11 p.m.? Answer: Assuming a constant rate of elimination, the cocaine concentration at 11 p.m. would be about 0.05 mg/L.*

0.20 mg/L → 0.10 mg/L → 0.05 mg/L at 11:00 p.m.

**Metabolism and chemical decomposition:** The fate of cocaine is associated with a series of enzyme-catalyzed and chemical reactions. The relationship between four of the major cocaine metabolites follows.



All of these metabolites exhibit less biological activity than cocaine. Cocaethylene is discussed below.

**The interpretation of premortem cocaine test results:** Most hospital-based drug test results for cocaine are done in urine by a method that is optimized for the detection of cocaine metabolite, benzoylecgonine, and a positive test result is not quantitative. Therefore, absent detailed review by an experienced toxicologist, a positive urine test result for cocaine metabolite(s) should only be considered as evidence of the last consumption of cocaine within 48 to 72 hours prior to the time of specimen collection.

**The interpretation of postmortem cocaine test results:** Because cocaine and metabolites of cocaine are subject to postmortem chemical decomposition, quantitative test results should be carefully interpreted. In some cases, this review should include the following:

- Prior or chronic use of cocaine including route of administration
- Time and conditions between death and autopsy
- Anatomic location of specimen collection
- Use of preservatives and storage conditions
- Time between autopsy and laboratory testing
- Testing process and procedure
- Cocaine and cocaine metabolite concentrations and concentration ratios
- Statements regarding the deceased's prior appearance-behavior-demeanor

**Absorption, distribution, elimination, and decomposition of cocaine ... measurement ... and extrapolation:** It is important to remember that the rise and fall of the measured blood cocaine concentration depends on the nature and time-course of drug consumption (e.g. what chemical form, by what route, in what amount, over what period of time was cocaine used) and the related features including the rate of absorption, the distribution and redistribution of cocaine between body tissues and fluids, and the rate of elimination ... while

extrapolation using these factors depends on reasonable assumptions regarding the decomposition of cocaine between specimen collection and testing as well as the accuracy of the test result(s).

### **Cocaethylene**

Cocaethylene is the product of a transesterification reaction between cocaine and ethanol.

### **Unique crack cocaine markers**

Smoking cocaine results in the formation of a pyrolytic product (methylecgonidine, MED) that is absorbed and rapidly converted to ecgonidine (ED). The selective extraction and detection MED and EC is currently considered an analytical marker for smoking cocaine. The quantitation of urine ED might prove to be a reliable marker for the active use of cocaine.

### **Another illustration ... Heroin:**

Heroin is diacetylmorphine. Intravenous administration is the usual route of abuse .

**Metabolism and chemical decomposition:** The post-absorptive fate of heroin is associated with a series of enzyme-catalyzed and chemical reactions.

### **Heroin (H) (Diacetylmorphine)**



### **6-Monoacetylmorphine (6-MAM) 3-Monoacetylmorphine (3-MAM) ?**



### **Morphine (M) → conjugation → M-6-G**

The conversion of heroin to 6-MAM and the conversion of 6-MAM to morphine is due to the sequential chemical hydrolysis of heroin's diacetyl group:

### **H → 6-MAM → M**

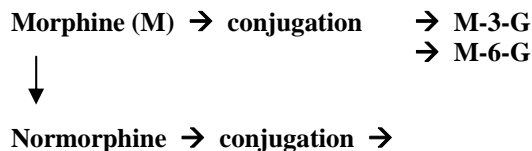
Following a fatal overdose of heroin, 6-MAM is sometimes detected in blood or urine or in tissue surrounding an injection site.

Absent the detection of 6-MAM or convincing evidence of heroin-related drug paraphernalia or reliable testimony, the presence of morphine is not necessarily reliable evidence of the prior use of heroin.

**Effects:** While postmortem findings such as pulmonary edema are consistent with a drug overdose, gross or microscopic findings are rarely specific for a heroin-related death. Therefore, an opinion regarding the cause and manner of death should include a consideration of all available toxicology test results, witness statements, medical and drug-related history, and relevant physical evidence.

## A final illustration ... Morphine and Codeine:

**Metabolism:** Metabolism involves an enzyme-catalyzed N-demethylation to normorphine and enzyme-catalyzed conjugation of morphine with glucuronic acid (M → M-3-G and M-6-G) and sulfate.



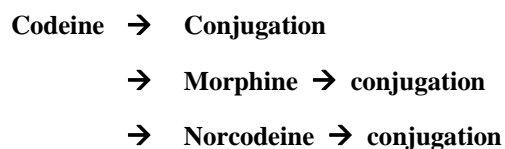
While M-6-G is biologically active, the total metabolic profile for morphine exhibits substantially less biological activity than the equivalent concentration of morphine.

When interpreting toxicology test results, it is very important to know if the morphine test results reflect *free* (i.e. unconjugated) morphine or *total* (i.e. conjugated and unconjugated) morphine.

### Codeine:

Codeine is a narcotic analgesic. It occurs naturally in opium or it can be produced by 3-O-methylation of morphine.

Metabolic studies have disclosed the following:



**Special notes:** *Positive urine codeine and morphine test results are not certain evidence of the abuse of any opiate including heroin. SAMHSA, NIDA, and other scientific agencies or organizations have long recognized that the ingestion of poppy seed products can result in high urine concentrations of morphine and codeine, and they have established guidelines for the interpretation of urine drug test results.*

*The pharmacokinetics of the distribution and elimination of individual opiates are more variable than many other drug classes. Disease states including cancer, liver disease, and renal failure have been associated with increased pharmacokinetic variability; and opiate concentrations achieved when treating chronic pain sometimes exceed levels thought to be fatal.*

## Therapeutic Drug Monitoring

*Therapeutic drug monitoring is a clinical tool that takes advantage of generally accepted relationships between drug dosage, drug concentrations in body fluids such as blood serum, and the expected clinical response.*

## Therapeutic drug monitoring ...

The ability to monitor the concentration of therapeutic drugs has dramatically influenced the field of clinical pharmacology and toxicology. The underlying rationale is that, for most drugs, the pharmacological effect tends to be proportional to drug concentration in extracellular fluids such as blood serum.

Clinical experience and pharmacological studies of dose-effect relationships have clearly demonstrated that measurement of drug concentration in blood serum yields a much better correlation with the desired clinical effect than does the total daily drug dosage.

### Therapeutic drug monitoring takes into account the following variables:

The patient's age, weight, height, sex, and general physical condition as well as significant injury or pathology affecting the patient's absorption-distribution-elimination-response to a drug

The route of drug administration, dosage form, and dose expressed as total drug weight or weight per the unit body mass of the patient (i.e. milligrams of drug per kilogram of body mass)

The effects of both drug-drug and drug-metabolites

The time-line relating to the introduction of drug therapy, change in drug dosage, or discontinuation of the drug and the resulting BDC

For example: If a drug such as phenobarbital is discontinued, the time required to realize the nearly complete elimination of the drug from blood serum is equivalent to about five or six times the drug's elimination half life. If the daily dosage for a drug is increased, it also takes the equivalent of about five or six half-lives for the drug concentration to reach its higher steady-state concentration.

The practical clinical use of therapeutic drug monitoring data (e.g. the use of a blood serum drug concentration test result) will consider patient factors, clinical response, and the generally accepted therapeutic range.

The generally accepted therapeutic range is the concentration range generally associated with an optimal relationship between risk and benefit to the patient.

For some drugs such as the antidepressant amitriptyline, the therapeutic range must include the biologically active drug (amitriptyline) plus the biologically active metabolite (nortriptyline). And, in some cases, one should pay special attention to the relative concentrations of drug and drug-metabolite as well as the potential for drug-drug interaction.

*A case vignette follows.*

**A case vignette:** Following a two car high speed MVA, one of the operators was transported to Kimble Community Hospital and then University Hospital, where his medical care and treatment included comprehensive blood serum toxicology testing. The results follow:

His Two-hour Post-MVA Test Results

Phenobarbital	5 mcg/ml
Phenytoin	ND (<5 mcg/ml)
Amitriptyline	1.92 mcg/ml
Nortriptyline	0.22 mcg/ml

An alcohol test was ordered but not reported.

His prior medical records indicated on-going treatment for a seizure disorder following an alcohol-related fall and chronic depression. No prior drug levels were documented.

*A very brief summary of the case analysis including the interpretation of alcohol and drug test results follows.*

**Phenobarbital and phenytoin**

The post-MVA phenobarbital level of 5 mcg/ml is less than the usual therapeutic range of 20-40 mcg/ml and indicated a subtherapeutic concentration at the time of the MVA. The results for phenobarbital and phenytoin are consistent with poor patient compliance following the last documented prescription refills 112 days prior to the MVA.

**Amitriptyline and nortriptyline**

The test results are consistent with a recent self-administered over-medication with amitriptyline and a total drug-and-metabolite concentration that is in the toxic range.

**Alcohol**

*As a result of on-going discovery, his blood serum alcohol concentration (BSAC) test result at Kimble Community Hospital was disclosed.*

*His one-hour post-MVA BSAC was 297 mg/dl.*

*The Kimble Hospital ER record indicated two i.v. sites (RAC and LAC, wide open) prior to admission; but his admission hematology test results included a normal hemoglobin and hematocrit. He was 6' tall and his recorded weight was 180 pounds.*

His BSAC of 297 mg/dl is the same as 0.297%; and a BSAC of 0.297% is equivalent to a whole blood alcohol concentration (BAC) of about 0.25%. The result indicated a total prior alcohol consumption equivalent to more than 15 ounces of 80-proof liquor and a BAC of at least about 0.20% at the time of the MVA.

At the time of the MVA, he was legally intoxicated, physically and behaviorally impaired, and at substantially increased risk of both MVA and an alcohol-induced seizure.

## Premortem Drug Testing

### Blood, blood serum, and urine

*Over 85 percent of the hospitals in the United States routinely offer only limited urine drug screening based on four to eight different immunochemical tests for specific drugs or their metabolites or drug classes representing only a small fraction of the potential drugs of abuse.*

### Hospital-based Patient Drug Testing:

#### Drug testing by immunoassay and the interpretation of test results

**Immunoassay Tests:** Immunoassays are the most frequently employed hospital-based test methods used to detect the presence of drugs or drug-metabolites in urine:

Chemical test reagents including a semi-synthetic antibody to a specific drug or drug-metabolite or drug-class are mixed with the test specimen (usually urine or blood serum); an antibody binds to a prototypical drug or drug-metabolite characteristic of a class of drugs; the binding of the antibody to drug or metabolite affects the binding of antibody to other chemical reagents; the effect of this competitive process is monitored using a selective physical or chemical reaction and automated an instrument such as a spectrophotometer; and standards or positive and negative control samples are used to establish a test “cut-off” for the presumptive identification of positive and negative specimens.

*Most hospital laboratories do not confirm positive drug test results ... but, the result are usually reliable..*

#### Test specimens-test reliability-results interpretation

**Urine:** The biological specimen most frequently used to test for prior drug use. The time-window within which the prior use of a drug would usually be detected depends on the frequency-route-extent-and-last use of the drug, the absorption-distribution-elimination of the drug or drug-metabolites, the date of specimen collection, the volume of specimen tested, and the sensitivity of the test method.

The most frequently performed urine drug test batteries include the following tests for a drug or class of drugs:

- Amphetamines
- Opiates
- Benzodiazepines
- Cocaine

**Test Reliability:** Performance characteristics are used to describe test reliability. Definitions of two of these performance characteristics follow:

**False positives:** Specimens that should test negative actually test positive.

**False negatives:** Specimens that should test positive actually test negative.

**Interpretation of a positive urine drug test:** A true positive urine drug test result means that the person consumed sufficient drug to account for the detection of a specific drug or drug-metabolite or a member of a class of drugs in the urine specimen. However, without case-specific assumptions or reliable evidence, a positive test does not establish . . .

- When the drug was last taken
- The route by which the drug was last taken
- How much drug was last taken
- The drug concentration in urine
- The drug concentration in blood
- The expected effect(s) on a person
- The relationship between drug use and some other event such as an accident

**Blood serum:** Very few hospitals follow the recommended practice of reserving blood or blood serum for possible quantitative analysis following interpretation of the urine drug test results. If this were done, a toxicologist might be able to estimate the time of last use and/or the amount of drug consumed and/or the expected toxicity associated with the drug use, and a toxicologist would be better able to consult regarding drug-specific therapeutic intervention.

Because the concentrations of drugs and drug metabolites in blood are usually lower than they are in urine and a much smaller volume of blood is usually collected for drug testing, qualitative blood or blood serum drug screening is less effective compared to urine drug testing. As mentioned earlier, the better approach would be to reserve blood for quantitative drug testing.

## Probationary Urine Drug Testing:

### Test reliability

**Immunoassay tests** are almost always used to test for drugs in urine. These are screening or presumptive tests . . . tests used to distinguish apparently positive specimens from apparently negative specimens.

**Confirmation tests** are rarely used to verify a positive screening test. Workplace drug testing is more rigorous.

### Points of comparison:

Workplace drug testing programs usually rely on well defined SOPs, the confirmation of positive results, MRO review, and the opportunity to request independent retesting. For example, federally mandated urine drug testing guidelines controlled by the Substance Abuse and Mental Health Services Administration (SAMHSA) include documentation of the following:

- Specimen collection procedures
- Specimen security and chain of possession

- Drug screening tests with pre-defined cut-offs
- Confirmation of positive results by GC-MS
- Medical/drug/results review by an MRO
- Split-specimen retesting options
- Laboratory quality assurance

## Postmortem Drug Testing

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### Postmortem specimens:

#### A good set of blood specimens

A set of blood specimens (e.g. from the femoral vein, some other peripheral vein, and the heart) allows for the best indirect estimate of a drug-related effect (usually based on the drug concentration in blood taken from the femoral vein) and the detection of unusual specimen:specimen drug concentration ratios that might be due to the i.v. injection of drugs, the absorption of high concentrations of drugs from the gastrointestinal tract, or the postmortem diffusion or redistribution of drugs resulting in higher, localized drug concentrations.

#### Urine

Urine is relatively “clean” and plentiful; and urine usually contains relatively high concentrations of drugs and drug-metabolites. Urine is a good specimen to use for initial screening tests. While drug concentration and drug:drug-metabolite ratio sometimes suggest when the drug was taken, the concentration of a drug in urine is not a good measure of drug-related effects.

#### Gastrointestinal contents

The inspection-recovery-testing of g.i. contents often allows for the determination of the type(s) of the drug(s) taken (e.g. pill v. capsule v. pulverized products), the minimum amount of drug taken (based on g.i. volume and drug concentration), and the time the material was taken (e.g. before or with or after the last meal).

#### Vitreous humor

Vitreous humor is relatively immune to the effects of postmortem decomposition and disequilibria that could affect the interpretation of the drug test result. When a drug is suspect, a good set of specimens would include blood, urine, and one other biological specimen.

#### Injection sites

The dissection and analysis of injection sites can sometimes detect concentrations of parent drug(s) consistent with recent i.v. drug use. Quantitative analysis of a control specimen is important.

#### Hair and nasal swabs

Head hair can be cut near the root, sectioned to reflect the time-line of hair growth, and tested to determine the use of some drugs and the apparent time-course of that drug use. A nasal swab can be used to test for cocaine or other drugs taken by nasal insufflation.

### Pills, powders, syringes, and residues

Identify pills and capsules by manufacturer code; determine if the remaining number of pills or capsules is consistent with the deceased prescription drug records and prescribed dosage; check with a toxicologist to ensure that each of the suspect materials would be detected by the drug test procedures employed by the laboratory; and, in some cases, determine the percent purity of drug powders such as cocaine, heroin, PCP, and amphetamines.

### Drug test results:

#### Qualitative test results

A qualitative drug test result only relates to the apparent presence (i.e. detection) or the apparent absence of a specific drug or drug metabolite or class of drugs. It is important to know the analytical or drug universe for the test procedure and the sensitivity or detection limit for each drug or class of drugs or drug metabolite(s).

Remember that a negative qualitative drug test result does not mean that the drug was not consumed. It only means that the drug was not detected and that, if the test is reliable, any drug consumption near to the time of specimen collection did not result in a detectable concentration of the drug or drug metabolite. It is important to know if-how-and-why the drug test universe and test sensitivity are relevant to drug-related case-specific questions.

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*An unspecified numerical test result reporting only an instrumental response does not mean that the result is the product of a reliable quantitative test ... and, it doesn't even mean that the result is a "good approximation" of the actual drug concentration.*

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#### Semi-quantitative test results

Semi-quantitative test results are approximations of the concentrations of chemicals in the test specimen. Semi-quantitative tests are less accurate than quantitative tests.

#### Quantitative test results

A case-specific assessment of quantitative accuracy is usually based on the review of laboratory documentation. When considering an evaluation of the accuracy of a quantitative test, you should ask an experienced toxicologist to discuss case-relevance, reported results, processes relating to results review, potential discovery requests, and options relating to retesting.

*When considering the accuracy of quantitative test results, retesting should usually be your last choice. Never retest prior to discussing your case with a toxicologist.*

## Drug Testing by GC and GC-MS

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### GC drug screening:

The objective is to detect drugs and drug metabolites based on extraction-and-concentration of drugs and metabolites, separation by gas chromatography (GC), and qualitative identification based in part on the GC retention time (RT). Specialized detector systems can be used to increase the specificity of the test. An example test scheme for GC drug screening follows:

A volume of internal standard (IS) with physical-chemical properties similar to the Target drug(s) of interest is added to a volume of the unknown specimen(s) and control or check sample(s);

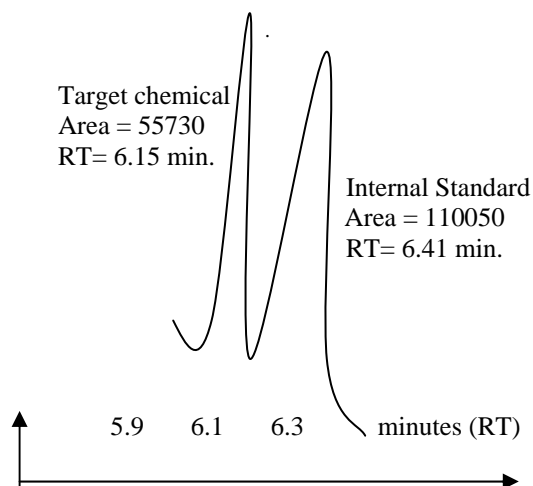
The pH (acidity or alkalinity) of the mixture is adjusted to optimize the extraction of a Target drug or group of drugs;

The samples are then extracted by mixing with an immiscible chemical solvent such a hexane;

The solvent extract is recovered and concentrated by evaporation; and

The concentrated extract is introduced into a GC.

The Target drugs and drug metabolites elute from the GC over time and are directly introduced into a detector system. The time between specimen introduction into the GC and detection is referred to as the retention time. Detector response is a very rough indicator of the relative amounts of the drug(s).



Sample Injection - Retention Time (RT) -

In this case, the absolute retention time (RT) for the Target compound is 6.15 minutes and the relative retention time (RRT) compared to the IS is about 0.94.

Tentative identification of an unknown or Target chemical can be based on a series of analytical features including the following:

Specificity of the extraction method

The RT or RRT compared to a data base

Selective detectors that respond to chemicals containing nitrogen-or-phosphorous or chlorine or the use of a mass spectrometer (MS)

The presence of other chemicals consistent with the expected metabolism of a suspect chemical

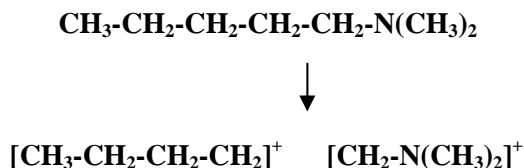
### GC-MS confirmation ... "the gold standard":

When a gas chromatograph (GC) is coupled with a mass spectrometer (MS), you can realize some very special advantages: The GC is used to separate the chemical constituents in a mixture and the MS is used to selectively identify and/or determine the concentration of individual constituents.

**GC.** A sample is vaporized in a sealed-heated-pressurized injection area such that the constituents are carried along with inert carrier gas into and through a column used to separate individual constituents based on physical-chemical properties such as volatility and chemical polarity ... properties that are a function of the chemical structure of the constituents. After a time called the retention time, the individual constituents exit from the column and are available for detection based on a variety of analytical-chemical characteristics including the passage of time between injection-and-elution (the retention time, RT), and/or the presence of a specific element or combination of elements such as nitrogen or sulfur, and/or "molecular fingerprinting" such as that accomplished by a MS.

**MS.** When interfaced with a GC, the individual (separated) constituents and inert carrier gas are introduced into the MS via a short, heated transfer system (interface) that also removes some of the GC carrier gas. Inside the MS, the chemicals that sequentially enter the MS source are subject to electron beam or other ionization process. As a result of ionization, individual chemical molecules are broken apart into charged fragments with different masses (based on the number of the different elements such as carbon, hydrogen, oxygen, nitrogen, in a molecular fragment); these ions or molecular fragments are separated in the mass analyzer portion of the MS; the fragments are detected; and the data are analyzed by a computer. This allows for the construction of a type of "molecular fingerprint" or mass spectrum for each chemical constituent eluted from the GC column. The review of this mass spectral data can allow for the determination of the constituent's molecular weight, the number of different types elements (C, H, O, N, etc.) in each molecule of the chemical, and the MS-"fingerprint" or fragmentation pathways associated with the ionization-fragmentation-

detection in the MS. The fragmentation or MS-fingerprints of some unknown constituents can sometimes be identified by matching with a computer data base. A simple ionization-fragmentation process follows:

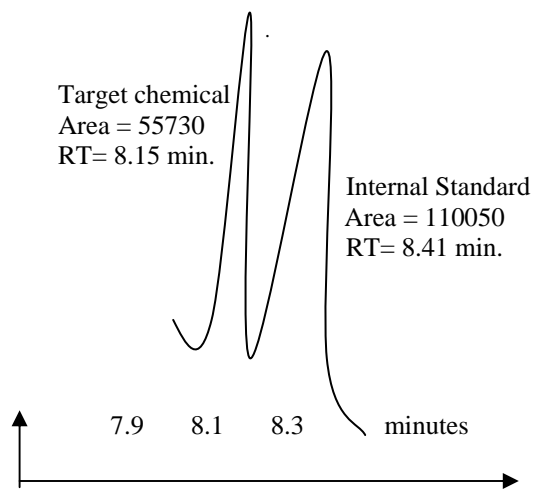


**The reliability of GC-MS.** While GC-MS is potentially molecule-specific and has sometimes been referred to as the "gold standard" of drug-detection methods, GC-MS based drug tests are not infallible:

*For a negative GC-MS drug test, there might be questions regarding specimen collection or post-collection adulteration or decomposition or questions regarding test sensitivity or laboratory error. For a positive test result, questions might include the rate of false-positive test results. For a true positive test result, there might be a question of whether or not the subject knowingly consumed the drug.*

### Quantitation by GC or GC-MS:

GC or GC-MS is used to separate-and-identify the Target chemical and the IS based on retention time (RT); and the MS detector response to one or more fragments of the Target chemical and IS in each sample is measured as peak area or as a summation of the MS response;

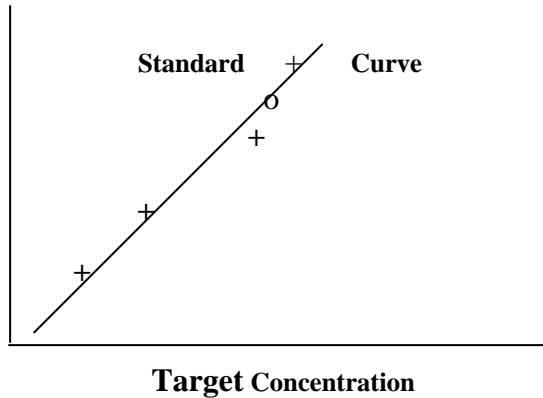


The peak area ratio for the Target chemical versus the internal standard is then calculated;

$$\text{Target/IS ratio} = 55730/110050 = 0.50$$

The peak area ratios or detector response ratios for the Target chemical and the IS (i.e. the Target:IS ratios) for a series of standards (+) are then analyzed by a micro-computer to prepare a standard curve; and

### Peak Area Ratio (Target:IS)

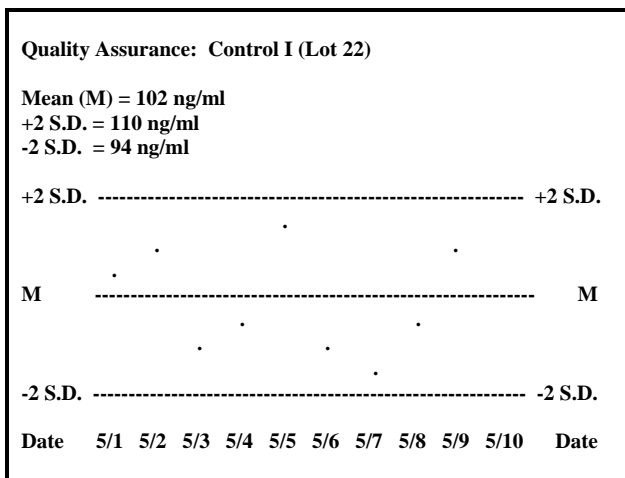


The Target:IS ratio for each unknown (o) is compared with the standard curve to determine the concentration of the Target chemical in the unknown.

Because the Target:IS ratio for each sample is established when the internal standard is added to the sample, the procedure is "self correcting" for many of the potential errors that would otherwise be associated with many other types of tests.

Unknowns are often run in duplicate or triplicate to determine reproducibility; and unknowns are also often separated by drug-free check samples in order to establish the absence of cross-contamination.

**Quality Assurance:** One aspect of quality assurance is the determination of the reproducibility of a test procedure; and one measure of reproducibility is the statistical deviation of test results for check or control specimens tested on different days. A typical day-to-day quality assurance chart follows.



Quality assurance is achieved as the result of a combination of factors. One laboratory practice is to require the review of test procedures such as standardization if the control result falls outside of two standard deviations (SDs) from the mean control value.

The standard deviation (SD) and the coefficient of variation (CV) of a test are two frequently reported measures of reproducibility. For a highly reproducible test, the day-to-day SD might be 4 ng/ml for a control with a mean of 102 ng/ml based on test results over a period of one month. Given a mean of 102 ng/ml, a SD of 4 ng/ml would be equivalent to a CV of four percent; and one would expect that about 95 percent of the day-to-day test results for the control would fall in the range of 94 to 110 ng/ml (equal to plus or minus two SDs from the mean).

But ... remember that a reproducible test is not necessarily an accurate test... it only means that the test gives similar results when the specimen is tested more than once ... it does not mean that the test results are correct. On the other hand, an accurate test gives a reliable result that is reproducible.

## The Effects of Drugs

### Drugs and Accident or Incident

#### Case-specific Questions:

- What are the toxicology test results?
- How reliable are these results?
- What do the results mean?
- What contributed to the accident?

#### Motor vehicle accident:

The use of antidepressants and opioid analgesics by older drivers was associated with increased risk of injurious motor vehicle collisions. *Epidemiology* 5(6):591-98 (1994)



Persons who use minor tranquilizers were 4.9 times as likely to be involved in serious road accidents as those who did not use tranquilizers. *Ref. to Skegg et al. in Am J Psych* 142(5):543

*Older individuals are also at greater risk of alcohol or drug-related fall.*

#### Behavioral Effects ... Aggression:

#### Cocaine-ethanol-cocaethylene-and-assault

As previously described, the consumption of cocaine and alcohol results in the formation of cocaethylene (CE or BEEE).

## Cocaine + Ethanol → Cocaethylene

The individual and/or additive effects of cocaine, CE, and ethanol have been associated with adverse behavioral effects including deviant or violent behavior.

**Other drugs that have been associated with aggressive behavior include benzodiazepines such as diazepam or Valium.**

### Theories relating to aggressive behavior

#### A Common Theory: Alcohol and Drugs

Alcohol-related theories of aggressive behavior are probably applicable to a consideration of the relationship between the use of some drugs and aggressive behavior.



**Behavioral theories include the following:**

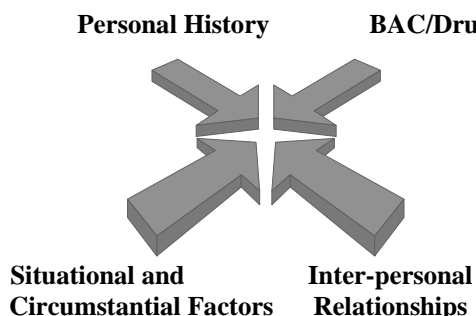
**Physiological disinhibition theory:** Alcohol/drugs increases aggression directly by depressing the brain center that normally inhibits aggressive behavior.

**Expectancy theory:** Alcohol/drugs increases aggressive behavior because people expect it to.

**Indirect cause theory:** Alcohol/drugs increase the probability of aggression as a result of cognitive effects that include reduced intellectual function. *A subject is less likely to avoid potentially troublesome situations or circumstances and/or the subject is less able to deal appropriately with such situations or circumstances.*

**Based on the uncertainties regarding the application of only one of these theories to a case-specific situation, it is prudent to consider all reasonable theories and relevant factors as part of a case analysis.**

#### Alcohol/drugs and Behavior: Case Factors



## Forensic Toxicology:

### Case analysis related to a suspect drug-related death

**Case Vignette:** As the result of a work-related accident, the deceased was permanently disabled and prescribed medication for chronic pain (narcotics), muscle spasm (cyclobenzaprine), and depression (amitriptyline). He was last seen by his wife about 12 hours prior to discovering his body on the family room couch. He was pronounced dead at the scene and his body was transported to the Office of the Medical Examiner. An autopsy was done 24 hours later.

#### Prior medical history

Prior medical history included concerns expressed by treating physicians regarding the deceased's dependence on narcotics and poor-compliance related to the prescribed use of antidepressants. Three physicians were identified and there was duplication in the prescriptions for narcotics.

#### Prior pharmacy records

24-month records from three local pharmacies were reviewed and compared with the available medical records and prescriptions. The pharmacy records indicated that three physicians prescribed narcotics (oxycodone and hydrocodone) in the 12-months prior to death. The records of one of these physicians were not included in the deceased's prior medical history. Discovery was expanded to include this physician.

#### Prior medical-pharmacy claims

This review disclosed claims related to one out-of-state pharmacy that provided oxycodone 11 months prior to death. A related prescription was never identified and discovery related to this prescription was not pursued.

#### Police report of death

The police report of the death investigation referred to a suicide note, concern regarding depression, and reference to a recent suicidal ideation. One empty prescription container for the most recent month's prescription of cyclobenzaprine was found in a waste basket located in the kitchen. Assuming that month's medication was used as directed beginning with the day it was refilled, 22 pills were missing.

#### Physical evidence

Physical evidence recovered by the police included six containers from pharmacies-of-record. The evidence was consistent with an impression that the deceased had accumulated narcotics as a result of *doctor shopping*.

#### Statements relative to the 24 hours prior to death

The deceased was last seen alive in the late evening hours ... about 8 hours prior to being discovered. He was described as being quiet but in generally good spirits.

### Statements of close friends or others

No other statements were obtained.

### Ambulance report

The EMS record indicated initial rigor consistent with death at least six hours prior to arrival.

### Death-related medical records

There was no emergency medical treatment.

### Autopsy report and related notes, etc.

The autopsy report included findings of pulmonary edema and pre-existing heart disease. Stomach contents, heart blood, and urine were submitted for toxicology.

### Toxicology test results

Stomach contents were positive for amitriptyline. Urine was not tested. Blood drug test results follow:

Amitriptyline	3450 ng/ml
Nortriptyline	1005 ng/ml
Oxycodone	120 ng/ml
Cyclobenzaprine	22 ng/ml

### Results interpretation

While the test results for the antidepressant (amitriptyline) and its metabolite (nortriptyline) were well in excess of the therapeutic range and potentially lethal, these postmortem drug levels could have been increased as a result of a perimortem disequilibrium or postmortem diffusion and/or redistribution. However, assuming a doubling of the actual premortem drug levels, the adjusted levels would be considered potentially lethal. Both amitriptyline and nortriptyline are potentially cardio-toxic and the subject of case reports of fatal cardiac arrhythmia.

Cyclobenzaprine is also potentially subject to a postmortem redistribution process, and the results were interpreted with caution.

Oxycodone is not thought to be subject to a postmortem redistribution process. The level was in the toxic range.

### Deposition of a knowledgeable person

The deceased's wife testified that her husband had not consumed oxycodone for at least two months prior to his death. She also testified regarding her discussions with her husband and his treating physicians regarding his apparent reliance on "pain medication".

## BRIAN E. PAPE, Ph.D., BCFE, BCFM

Dr. Brian Pape is the principal consultant with Pape & Associates, specializing in toxicology and related sciences. His professional experience includes the following:

- Associate Professor of Pathology (*Clinical Appointment*), University of Massachusetts School of Medicine.
- Senior Associate Consultant for Mayo Clinic (Rochester, MN) and Director of Toxicology at New England Toxicology Services (Woburn, MA).
- Director of Toxicology and Associate Professor, Department of Pathology, University of Missouri School of Medicine.

Dr. Pape has published more than 50 research papers, abstracts, and professional articles relating to alcohol and drugs, pesticides and toxic chemicals, analytical chemistry, forensic science, and general toxicology. He authors the *Toxicology Reporter*.

He has served as a technical and expert consultant to business, labor, and governmental agencies. He has been qualified as an expert in toxicology and related sciences in State and Federal Courts.

Dr. Pape has been board-certified by the American College of Forensic Examiners (BCFE) and the American Board of Forensic Medicine (BCFM).

He has been qualified on more than 100 occasions in State and Federal Courts. His case testimony has included liquor liability, alcohol and drug related testing-effects-and-accidents, laboratory testing, toxic torts, and product liability.