Principles and Procedures in Forensic Toxicology

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KEYWORDS
• Review • Drugs • Analysis • Forensic • Toxicology • Procedures

KEY POINTS
• Forensic Toxicology is composed of Postmortem Toxicology, Human Performance Toxicology and Drug Urinalysis.
• Forensic Toxicology results have the potential of being scrutinized in court; as a result, testing is more comprehensive, with greater emphasis on specificity and accuracy in identifying potential toxicants.
• Conclusions about postmortem results must be made after considering all aspects of a case, including medical records, matrices analyzed, drug interactions, drug tolerance, postmortem interval, and the like.

INTRODUCTION
Forensic toxicology concerns the application of toxicology to situations that may have medicolegal review, and as a consequence, results must stand up to scrutiny in a court of law.1 There are primarily three subdisciplines of forensic toxicology:

1. Postmortem toxicology, more recently referred to as death investigation toxicology.
2. Behavioral or human performance toxicology, which concerns
   a. Impaired driving as a result of alcohol and/or drugs consumption.
   b. Drug-facilitated sexual assault cases.
   c. Doping control. Screening of athletes for performance-enhancing substances is monitored by the World Anti-Doping Agency.2 In this category must be included equine and canine toxicology testing, because entire laboratories are dedicated to this specific purpose.
3. Forensic workplace drug testing or drug urinalysis, which is performed as a preemployment and/or random monitoring of employees for illicit drugs or court-ordered testing of convicted drug offenders.

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Closely related but in a category of its own is forensic drug chemistry. This discipline is concerned with drug and chemical analysis, as is toxicology, but in regard to nonbiological specimens such as seized bales of marijuana, packets of synthetic cannabinoids, pills, “meth lab” reagent analyses, rocks of crack cocaine, and the like. Toxicology and drug chemistry laboratories often work together on different aspects of a case, especially when the laboratories are housed in the same facility. An important consideration when laboratories are in the same building is that extraction of drugs by each discipline must be accomplished in different rooms to avoid possible contamination of toxicology specimens by the relatively massive quantities of drug chemistry specimens.

To assist with the practice of forensic toxicology, a guide as to how the discipline should be performed is provided in the form of forensic toxicology laboratory guidelines, prepared by the Society of Forensic Toxicology (SOFT) and the Toxicology Section of the American Academy of Forensic Sciences (AAFS). Also, at the time of this writing, a “draft” document entitled Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology has been released for public comment. The practice of urine drug testing is defined by Mandatory Guidelines for Federal Workplace Drug Testing Programs, issued by the Department of Health and Human Services. This review is restricted to the subdisciplines of postmortem and behavioral toxicology.

**HOW FORENSIC TOXICOLOGY DIFFERS FROM CLINICAL TOXICOLOGY**

Clinical toxicology is typically hospital-based, with the emergency room physicians being the primary customers. Analytical results must be obtained with speed to help confirm the therapeutic regimen for living patients, and an initial screen result by itself is sufficient for use in medical evaluation. Clinical analysts rarely are called about judicial matters, compared with forensic analysts. Quantification and confirmation of drug findings are not usually relevant to the treatment and often are not possible because of time constraints. Analytical instrumentation such as immunoassay provides fast but less specific results at a relatively lower cost. In larger laboratories with high-end analytical capability, physician-ordered therapeutic drug monitoring is performed.

By comparison, forensic toxicology can move at a slower and more comprehensive pace. Critical to the analytical process is a rigorous chain of custody from which the identity of the specimen is certain. Because the results for any case have the potential to end up in a court of law, there is a greater emphasis on specificity and accuracy in identifying potential toxicants. The cost of analysis is generally higher. For results to be reported, both a screen and confirmation must be performed using two different analytical procedures wherever possible; alternatively, when two analytical procedures are not available, a confirmation may be accomplished using (a) two different methods for extraction, (b) by demonstrating that a specific drug (metabolites) is present in two different specimens (eg, blood and urine), and/or (c) the drug is listed as part of the case history from the standpoint of medical record, prescription record, or death investigation.

**CERTIFICATION AND ACCREDITATION**

Forensic toxicology and all fields of forensic science are currently experiencing a period of accelerated change. The impetus for change came in 2009 with the National Academy of Sciences report on the need for overhaul of forensic sciences in the United States. A major recommendation of this report was that forensic scientists
should be certified in their specific discipline and laboratories should be accredited. Forensic toxicology certification is available primarily through two organizations: The American Board of Forensic Toxicology (ABFT) and the Forensic Toxicology Certification Board. Other certifications include the American Board of Clinical Chemistry, the American Board of Toxicology, the American Society for Clinical Pathology, and the National Registry of Certified Chemists. In addition to certification of analysts, certain states require that individuals performing toxicology testing on specimens from impaired drivers be licensed by the state’s health departments.

Accreditation of toxicology laboratories can be accomplished through The ABFT and/or, based on the ISO 17025 Standards (Testing and Calibration Laboratories), through the American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD/LAB) program or Forensic Quality Services, Inc. Currently New York, Texas, and Oklahoma are the only states that require that toxicology laboratories be accredited. Other accrediting bodies include the National Laboratory Certification Program under the Substance Abuse and Mental Health Service Administration, the National Association of Medical Examiners (NAME), and the College of American Pathologists (CAP).

**TOXICOLOGY TESTING**

**Chain of Custody**

In that the results of forensic toxicology testing may be used in court proceedings, the first necessary component of the testing process is to demonstrate the validity of test specimens. This demonstration is accomplished through the chain of custody, which documents the chronologic disposition and condition of specimens from the time of collection to the time of disposal. The person initiating the chain of custody would typically provide the identity of the individual from whom the specimen was collected, what the specimen is, when it was collected (time and date) and by whom, including signatures. Tamper-evident tape with initials across the tape may be used to help maintain the integrity of the specimen. As the specimen moves through the transfer and testing process, printed names and signatures of releasing and receiving persons are recorded as are the time and date, the condition of the specimen, and the reason for transfer. Without correct, legible, and intact chain of custody documentation, the integrity and security of the specimens cannot be established, and the results of toxicology testing may be judged to be inadmissible to the court. The chain of custody form is often combined with the toxicology request form into a single document. The toxicology request form allows the selection of specific testing batteries such as a volatile screen, drug of abuse screen, comprehensive analysis, or other special testing requests.

**Testing Service Provided**

A standard testing battery within a forensic toxicology laboratory will include an alcohol (volatile) screen, a drug of abuse screen, electrolyte profile, and a comprehensive analysis. All screens include confirmation and quantitation of any positive results. Volatile analysis is most commonly performed by gas chromatography (GC), whereas drug of abuse screens are performed with immunoassay. Immunoassay is continually expanding to allow screening for tricyclic antidepressants, salicylate, acetaminophen, methadone, oxycodone, barbiturates, carisoprodol, promethazine, and other drugs. Some laboratories are transitioning to use of high-performance liquid chromatography (HPLC)/mass spectrometry (MS) for drug of abuse screens. Confirmations are most often performed by GC/MS analysis with or without derivatization of analytes or with HPLC/MS/MS, if this instrument is available. A comprehensive
drug screen will include a volatile assay, an immunoassay of blood and urine, followed by GC/MS analysis of extracts of blood and urine. Electrolyte analysis is performed on vitreous humor rather than blood. Electrolyte values in blood are difficult to interpret after death, whereas sodium, chloride, magnesium, calcium, urea nitrogen, and creatinine remain essentially unchanged in the early postmortem period in the vitreous. Elevated glucose will persist in the vitreous allowing detection of hyperglycemia, whereas normal glucose levels fall and have no interpretive value. Other analytical procedures provided by laboratories fall into the category of “caseregistered” testing. These are tests that are not routinely performed but are necessary based on the circumstances of the case. Examples include (1) carboxyhemoglobin assay in victims from house fires or decedents found in automobiles, (2) antipsychotic batteries for schizophrenics and manic/depressives, (3) anticonvulsant testing for seizure histories, (4) heavy metal assay for alleged poisonings, (5) gamma-hydroxybutyric acid in drug-facilitated sexual assault cases, and ethylene glycol as indicated. To complete case analysis, evaluation of evidence (patches, contaminated clothing, pill from gastric contents, drug paraphernalia (spoons, syringes, glass mirrors, wire mesh, and so forth) for drugs should be performed. If toxicology and drug chemistry are housed in the same laboratory area, evidence items can be analyzed by drug chemistry.

**Matrices**

The typical matrix for workplace or court-ordered drug testing is urine, although use of alternative matrices (sweat, hair, and/or saliva) are being examined to with increasing frequency. Behavioral toxicology most frequently is performed on blood and/or urine specimens. Blood for forensic analysis is collected in gray top tubes containing the antimicrobial additive sodium fluoride and potassium oxalate as an anticoagulant. In that results may be presented in court, collection of blood in gray top tubes is an important forensic consideration. An often used defense strategy is to question the integrity of the specimen from the standpoint of in vitro production of ethanol. This argument is moot if the specimens are collected in gray top tubes and refrigerated. Interpretation of impairment from drug levels in urine is not possible, although some states have per se driving laws based on the concentration of drugs in urine. Whether the individual was under the influence of drugs or not cannot be established from urine results; all that can be reasonably known is that the individual was exposed to the drug.

**Postmortem**

Postmortem specimens are many and quite varied. If only an external examination is performed (no autopsy), then blood, urine, and vitreous humor are typically collected. During an autopsy, blood, urine, vitreous, bile, gastric, liver, spleen, kidney, brain, muscle, and hair may be collected. For fluids that have a finite volume such as gastric, bile, and urine, it is important to record the total volume present so that the total amount of drug can be calculated for the specific compartment. Other specimens that may be collected depending on the condition of the body and the toxicant of interest include nails, bone, meconium, cerebrospinal fluid, and blood clots. If the clot is connected to the cause of death, such as traumatic head injury with subdural hematoma, the clot should be homogenized and analyzed. This procedure will give some indication of blood constituents immediately following the time of the injury.

**Hospital Specimens**

Considerations for blood clots are similar for hospital specimens. If the decedent arrived at the hospital and admission specimens were collected, these are the most
important specimens to analyze to obtain a valid interpretation of drug or alcohol contributions to the cause of death. Conversely, if admission specimens are not available and the hospital record reflects an extended hospital stay and/or transfusion/hemodilution, performing toxicology on postmortem specimens may be judged to be of little value. If family members indicate that they believe the hospital caused their loved one’s death, then it is important that the laboratory analyze both admission blood (if available) and postmortem blood. Most hospitals keep collected specimens for a short time (eg, 1 week) before they are discarded. Blood banks may hold specimens for a longer period than the hospital. Death investigators are trained to act quickly to recover hospital specimens, if they are available. In some situations, hospitals may work with coroner/medical examiner offices to preserve blood when a medicolegal investigation is expected.

Other Analytical Issues for Consideration

1. **First assay.** The first test performed in a forensic analysis should be a screen for volatiles, in that ethanol, acetone, methanol, and other volatile constituents will be depleted in concentration each time the stopper is removed from the blood, vitreous, or urine tube.

2. **Dedicated instrumentation.** If the laboratory can afford the luxury of instruments dedicated to specific assays, this is an efficient way to operate. Time spent changing columns and loading new methods is avoided so that the flow of analytical assay is more productive.

3. **Drug stability.** Certain analytes (olanzapine, promethazine, chlorpromazine, chlorprothixene, thioridazine, trifluromazine, ziprasidone, zopiclone, cyanide, synthetic cathinones, cocaine) should be measured as soon as possible because of instability in storage. It is a good practice to characterize the stability of analytes as part of the validation of the analytical procedure. In most cases, stability is improved by refrigeration or freezing and by use of gray top tubes containing sodium fluoride.

4. **New drugs.** Laboratories must stay current with the constantly changing menu of available drugs, both prescribed and illicit. This ongoing task can be readily accomplished by attending annual meetings (SOFT, AAFS, The International Association of Forensic Toxicologists, NAME) and subscribing to relevant journals (*Journal of Analytical Toxicology* and the *Journal of Forensic Sciences*). SOFT’s quarterly newsletter (*Tox Talk*) is an online publication containing a “New Drug” section.

Additional requirements are

a. To obtain new drug standards. Commercial drug companies include Cerilliant, Lipomed, Cayman Chemicals, and Grace Chemicals, to name a few. If a drug is not available it can sometimes be obtained by contacting the pharmaceutical company that manufactures the drug.

b. GC/MS libraries must be updated continually. Commercial libraries appropriate for drug analysis including PMW (Pfleger, Maurer, Weber), NIST (National Institute of Standards and Technology), and Wiley Registry are available but expensive to acquire. Additional sources that are currently free are the RTI International “Forensic DB” (adopted from the AAFS library made available by Dr Graham Jones), Scientific Working Group Drug library, and of course, creating in-house libraries from acquired standards.

5. In GC and liquid chromatography (LC) analyses, to detect the possibility of carryover from one injection to the next, it is good practice to insert solvent blank
injections between control and case extracts. With autoinjectors, this process is easily accomplished using the instrument sequencing program.

PRINCIPLES OF QUALITY ASSURANCE

A quality assurance (QA) program is required to ensure that the laboratory produces consistently reliable drug/chemical identification and quantitation. Aspects of a QA program include

1. Competent analyst with access to continuing education. The number of analysts must be sufficient to handle the workload and provide testing services requested. If resources allow it, each analyst would ideally attend a national or international meeting each year. Certification in forensic toxicology should be encouraged. Subscription to relevant journals and acquisition of up-to-date toxicology texts are needed.

2. An adequate work environment with properly maintained instrumentation. Documentation of maintenance and corrective actions is required. A planned, systematic replacement of obsolete instruments should be incorporated into the laboratory budget.

3. Appropriate documentation of policies and procedures is required. This documentation is usually maintained as standard operating procedure manuals for administrative policies, quality assurance, analytical methods, accessioning of specimens (and disposal), training, safety, corrective actions, improvements, personnel training and competency, and the like.

4. Proficiency testing for volatile analytes and for drugs in blood, serum, and urine specimens is required. Typically, laboratories will subscribe to the CAP proficiency tests, which will include series AL1 (American Association for Clinical Chemistry/CAP whole blood alcohol/ethylene glycol/volatiles, or AL2 (same but in serum), and forensic toxicology–criminalistics (FTC) and toxicology (T)). These series require that the laboratory conduct proficiency testing three times each year for each series. For accreditation by the ABFT the AL1, FTC, and T series test must be performed.

5. Validation of new analytical methods prior to their use in case work may include the following criteria:
   a. Limit of detection (LOD), which is the lowest concentration detectable above background noise; usually three masses are used for confirmation.
   b. Limit of quantitation, which is normally the lowest concentration determined for the calibration curve.
   c. Linear range for an analyte is needed to establish the upper and lower concentrations at which the instrument response directly corresponds to the concentration of the drug/chemical. Determinations that exceed the linear range of the calibration curve must be repeated after the specimen has been appropriately diluted. Values that are less than the lowest concentration of the calibration curve, and greater than the LOD, are normally reported as less than that concentration or as “trace.” Nonlinear relationships (eg quadratic) may exist for specific analytes, and use of instrument software to find the “best fit” curve will improve analytical accuracy.
   d. Accuracy is assessed through the use of controls. In order to validate the calibration curve, control concentrations must be analyzed. Preparation of controls should be from a source different from the source used to prepare the calibration curve, or else the curve will most assuredly be validated whether it is accurate or not. As much as is possible, calibrators and controls should be “matrix matched” (prepared in drug-free blood or urine). Depending on the assay, an acceptable accuracy may be as much as ± 20% from the target value. Volatile analysis of ethanol should not vary more than ± 5% of the
target value. Along with positive controls it is imperative that matrix-matched negative or blank controls be run with each assay. Calibration curves should be prepared with at least three different concentrations that define the linear range (low, middle, and high). Controls should be prepared at both high and low concentrations relative to the calibration curve, and both high and low controls should be included at the beginning and at the end of a batch analysis. A good practice is to include calibrations curve and control samples with each batch analysis, although some laboratories may make use of “historical” curves and freshly prepared controls.

e. Precision of the assay is measured as “within run” and “between run” and will involve determining the coefficient of variation (CV) for results from 10 to 20 separate assays. A CV of 10% or less is generally acceptable.

f. Specificity: interference from other analytes (those commonly identified in specific assays) should be determined by analyzing target analytes with and without other drugs present.

g. Recovery or extraction efficiency is determined by analyzing a known amount of analyte with and without extraction. The extraction efficiency will vary depending on the type and condition of the matrix. Decomposed specimens will severely decrease the analyst’s ability to extract drugs from a matrix. Use of an appropriate internal standard should compensate for the variability in extraction efficiency. The best practice is to obtain deuterated homologs of specific analytes for use as internal standards. Laboratory resources may limit how many different deuterated internal standards can be used, because they are expensive to acquire.

h. Stability of analytes is often overlooked or not performed for need to place the assay in service. Knowing how long analytes will persist at the same concentration in a specific biological matrix is an important criterion that ideally, at some time, will be assessed.

i. Measure of uncertainty is a relatively new concept to forensic toxicology and is being progressively implemented in laboratories across the country. The need for determining uncertainty measurement for toxicology results was formally realized during a suppression hearing in Washington State. Accreditation by the ASCLD/LAB-International Program requires that uncertainty measures be known for specific analyses. Uncertainty measurement is how one determines the amount of variability that exists with a measured result, realizing that the true uncertainty can never be known. To determine what the uncertainty is requires identification of the most probable sources of uncertainty throughout the analytical process, followed by the formulation of an uncertainty budget. As an example, an uncertainty budget may be developed by considering the following:

- Reproducibility
- Purity of standards and reference materials
- Volumetric measurements as a function of maximum permissible errors and temperature
- Variability of calibrator solutions with temperature corrections
- Variability of pipette calibration
- Variability internal standard/sample delivery

Other uncertainties that are less significant than those listed previously may include

- Storage conditions
- Instrument effects
- Sample/matrix effects
- Computational effects.
The standard uncertainty is first calculated for each component of the budget. Different calculations must be applied to different types of uncertainty. Guides for these calculations are available.26–30 Once standard uncertainties have been calculated, the combined uncertainty is calculated by squaring the value of each standard uncertainty, adding these together and then taking the square root of the sum of the squared uncertainties. When calculating the combined uncertainty, if the individual standard uncertainties for budget item is less than one-third of the maximum standard uncertainty, it can be ignored.

The final uncertainty determination is made by calculating the expanded uncertainty. This calculation requires a designated confidence interval of 95% or 99.7% (two or three standard deviations). The confidence interval is represented by 2 or 3, respectively, which is called the coverage factor “k.” The expanded uncertainty is equal to the combined uncertainty multiplied by the coverage factor desired. The ASCLD/LAB-International Program is considering requiring that uncertainty measurement values be included on all toxicology reports, along with the measured value for the analyte.

An excellent reference for further reading on quality assurance is provided by Bramley and colleagues.30

POSTMORTEM INVESTIGATION

A strong forensic axiom is that no aspect of a death investigation is performed in isolation. There is communication with all staff members through a morning “viewing meeting” to discuss case history, medical records, pharmacy records, and the circumstances of the death. An important consideration is that “results of toxicology testing have to make sense” in light of the entire case findings. For example, GC/MS analysis of specimens and identification of cocaine with no detectable cocaine metabolites (benzoylcegonine, methyl ecgonine, cocaethylene) should give the toxicologist pause, in that cocaine does not present by itself in postmortem specimens. If this result occurs, the results of immunoassay screen for cocaine should be examined and a repeat analysis should be considered. Another example is a vitreous glucose greater than 600 mg/dL will often coincide with acetone in the blood and vitreous volatile analysis, because acetone is a product of diabetic ketoacidosis. An elevated glucose and acetone are complementary results. As is discussed later, heavy lungs following an overdose of central nervous system (CNS) depressants is an expected pathologic outcome.

Postmortem Interpretation of Toxicology Results

How does one determine that a drug concentration is therapeutic, toxic, or lethal? Unlike experimental toxicology, forensic toxicology must rely on case reports provided in the literature and on the experience of the toxicologist/pathologist. Many useful references are available to help guide the interpretation of postmortem drug levels.31–36 An important aspect for interpreting toxicology results is knowledge of the specific case history, the medical record, and record of prescribed (or unprescribed) medications. Which forensic professional is responsible for drug interpretation will vary depending on the coroner or medical examiner office. The pathologist may rely on the toxicologist to provide the interpretation, or restrict the toxicologist to providing only the identity and concentration of drugs. The best practice is for the toxicologist and pathologist to have an open dialogue and collaborate on the interpretation of toxicology findings. In addition to the drug concentration in postmortem specimens, other factors to be considered are drug-drug interactions, the site
Signs of Toxic Pathology

Before toxicology testing begins, there may be evidence that a decedent was exposed to certain toxicants based on findings at the external exam or from gross or microscopic findings at autopsy. Examples include drugs in pockets of clothing, pill fragments in the mouth or nose, powder around the nostrils, and transdermal patches on the body or in the mouth. Examples of pathologic signs are a reddish hue to the skin produced by carbon monoxide; hemorrhagic gastritis, which could have been caused by heavy metals (arsenic or iron); cyanide, which also produces a rosy hue to the skin and may be detected by smell by approximately 20% to 40% of the population at a threshold of 0.2 to 5 ppm; Mees lines (white lines across the nail beds of fingers and toes), which may be produced by arsenic and thallium along with a hyperkeratosis of the palms of the hands and soles of the feet; Burton’s lines (blue or black lines along the margin of the gum and teeth), signs of allergic reactions such as a swollen tongue, rash, swollen airway, and voluminous lungs, which should prompt a request for test for β-tryptase levels and serum immunoglobulin E; and fresh needle punctures and/or old track marks of the skin, which suggest intravenous drug abuse.

Postmortem Redistribution

The propensity for certain drugs to have artifactually elevated concentrations in heart blood following death is known as postmortem redistribution (PMR). Because the drug concentrations are not representative of blood levels when the individual was alive, use of drug concentrations in heart blood for interpretation can lead to erroneous conclusions about the cause of death. As the time interval between death and blood collection increases, the concentration of drug in heart blood increases. Blood collection sites farthest from the central compartment organs will be least affected by PMR. For purposes of interpretation, the preferential order of collection is femoral, then iliac, then subclavian vessels, and then heart. Drug levels in blood from the latter two collection sites are best left uninterpreted.

The phenomenon of PMR can be best characterized by thinking about drug distribution when a person is alive. Certain drugs are sequestered by central compartment organs (liver, lungs, and heart) at intracellular concentrations higher than the circulating blood. Examples of drugs that exhibit high levels of PMR are tricyclic antidepressants (e.g., amitriptyline) and the analgesic propoxyphene. Antemortem concentrations of these drugs may be greater than tenfold higher in liver compared with circulating blood levels. To maintain this concentration gradient during life requires energy and integrity of cell membranes. Cell death results in a loss of energy (oxidative phosphorylation of adenosine triphosphate ceases) and cell membranes are not maintained.

Determination of Dose in Postmortem Specimens is a Poor Practice

In clinical toxicology or therapeutic drug monitoring it is possible to calculate a dose consumed based on blood concentration, body weight, and the volume of distribution ($V_d$) of the drug as shown:

$$ \text{DOSE} = \text{Body Weight} \times \text{Conc} \times V_d $$

In drug overdose cases it is natural to ask how many pills or capsules were consumed by the decedent. If prescription bottles are available, this number may be
determined by counting the pills left in the bottle and correlating the pills consumed with how they were prescribed. To attempt to calculate dose after death based on postmortem blood concentration is a poor practice\textsuperscript{44} for the following reasons:

1. \( V_d \) is never known for a specific individual and varies depending on body type (body mass index).
2. Whether a drug concentration is at steady state is not known (is distribution complete?).
3. PMR may elevate drug concentration from two- to tenfold.
4. \( V_d \) is based on plasma/serum concentrations, and postmortem toxicology is performed on whole blood.

The fallacy of calculating pill counts should be pointed out to any pathologist who has to respond to questions from family members and/or law enforcement. One should never present postmortem pill counts as evidence in court.

**Cases of Suspected Overdose**

Confirmation of suspected drug overdose cases should be accomplished using additional specimen analyses. If a lethal level of drug is determined for blood, then analysis of liver will demonstrate whether the high level is a systemic intoxication. Not following with a confirmation of systemic toxicity can sometimes lead to the wrong conclusions, such as when an implantable drug delivery system (drug pump) continues to release drug after death.

**Tolerance or Loss of Tolerance**

When evaluating drug levels in a deceased individual, it is important to review the history and/or medical records to determine the degree of tolerance, if any, that might have existed. Tolerance is an acquired phenomenon as a result of continued exposure to a chemical substance. Mechanisms of tolerance may include (a) an increase in metabolism of a drug as a result of metabolic enzyme induction and (b) desensitization of the receptor with a decrease in pharmacologic response. Individuals in a methadone maintenance program will have levels of methadone exceeding 1 mg/L,\textsuperscript{31} whereas naïve users can expire from blood levels less than .2 mg/L. Whether tolerance to opiates, alcohol, or other drugs exists should be established before a drug level is ruled an overdose. Conversely, a loss of tolerance (opiates) by persons entering a drug rehabilitation program may result in their death if when they leave the program they resume their addiction at the same level as before they entered rehabilitation.

**Conflicting Causes of Death in Litigated Cases**

If the toxicologist’s report provides an interpretation of the drug level detected (therapeutic, toxic, or lethal), it is always important to review the case history as to how the individual died. Even though a high level of heroin or cocaine might be considered lethal and a reasonable cause of death, reporting the drug as lethal could pose a problem if the immediate cause of death was a gunshot wound to the head and the case is a homicide. Reporting the drug level as lethal under these circumstances would provide the perpetrator a ready-made defense.

**Specimen Collection in Decomposed Bodies**

Depending on the state of decomposition, specimen collection from decomposed bodies can be quite limited. The traditional specimens of blood and vitreous are the
first fluids to be lost; if the temperature is warm, these matrices may be gone after 48 hours decomposition. Other available specimens to be collected may include liver, kidney, muscle, bone, hair, nails, insect larvae, and/or soil beneath the remains. Liver, the traditional matrix used for interpretation of drug levels when blood is not available, should not be used for drug quantitation if the body is badly decomposed. After a few days decomposition, drug levels in liver can increase several-fold higher than what would have been measured at the time of death. This change is a result of anatomic location (adjacent to the gastrointestinal tract) and fluid loss that decreases liver mass. This dramatic increase in liver drug concentration can easily lead to erroneous interpretation as to cause and manner of death. Drug concentration in muscle changes much less over time than that of liver and may be a more reliable matrix for interpretation.

Drug-Drug Interactions

Although there are many different categories of drug-drug interactions that one could describe (such as drugs and dietary supplements or drugs and food), the three principal interactions involve

1. **Induction and inhibition of metabolism.** Detoxification of drugs and chemicals in the body is carried out by reactions known as phase I and phase II metabolism. Phase I mostly involves oxidation and, to a lesser extent, reduction or hydrolysis of substrates, whereas phase II concerns the conjugation of substrates with small molecules such as glucuronide, glutathione, sulfate, glycine, acetate, and others. Induction or inhibition of metabolism of one drug by another is almost always a result of phase I enzymes, principally hepatic cytochrome P-450 mixed function oxidases. A large body of drugs function as substrates, inducers, and/or inhibitors of this enzyme system. One drug inducing the metabolism of another drug, by causing the production of more enzyme, may decrease the efficacy of the metabolized drug. Conversely, inhibition of cytochrome P-450 metabolism by a drug can cause an increase in toxicity of another drug. There are primarily five families of cytochrome P-450 found in humans. Within these families, CYP3A4, CYP2D6, CYP2C9, CYP2C19, CYP1A2, and CYP2E1 are of importance. Keeping track of which drugs serve as substrates, inducers, and/or inhibitors for which isozyme can be a daunting task. However, knowing that the vast majority (~ 75%) of drugs are metabolized by only two isozymes, CYP3A4 and CYP2D6, helps to simplify; also that ethanol, in addition to alcohol dehydrogenase, is metabolized by CYP2E1. An in-depth discussion of cytochrome P-450 metabolism is outside the scope of this article. Suffice it to say that interpretation of postmortem toxicology results requires thinking about, and sometimes investigating the possibility of, induction or inhibition of metabolism of a drug by the presence of another drug. For the most part, however, drug-drug interaction as a result of induction or inhibition of metabolism is less of a concern with most drugs because the change in the drug concentration is typically small, and most drugs generally have a wide margin of safety and/or have multiple routes of metabolic elimination.

2. **Disruption of drug storage reservoirs.** The primary sites of drug storage are plasma proteins (albumin and acidic glycoproteins) and tissue (organs, muscle, and fat). If drugs bind to plasma proteins and/or tissues, they are no longer available to cause their pharmacologic effect. When another drug with a greater binding affinity for the binding site is introduced, the bound drug will become free to act. An example of toxicity as a result of drug-drug interaction is when ibuprofen is taken by a patient who is on warfarin. Use of ibuprofen (or other nonsteroidal
antiinflammatory drugs) with warfarin is contraindicated because ibuprofen will increase the concentration of warfarin and potentially cause unwanted hemorrhage. Therefore, some appreciation for the propensity of a drug to displace another drug from plasma or tissue proteins can be gained by consulting the percent protein binding data.

3. **Additive (synergistic) effects.** The most frequent type of drug-drug interaction that is encountered in postmortem, as well as human performance, toxicology is increased toxicity because of additive or synergistic effects. A pharmacologic effect common to numerous drug classes is CNS depression. CNS depression occurs as a direct or indirect result of consuming alcohol, opiates, barbiturates, benzodiazepines, tricyclic antidepressants, muscle relaxants, antihistamines, or lithium. Within these drug classes are as many as a hundred different drugs, so the likelihood of an individual consuming more than one CNS depressant is very possible. Individuals dying from depressant polypharmacy experience CNS depression, which leads to cardiac and respiratory depression (brain stem), followed by pulmonary edema (lung weight increases from a norm of 350 g to >1000 g) and death. Evidence of pulmonary edema may be visible at the death scene in the form of a “foam cone” on the nose and mouth. If a CNS depressant overdose is suspected as the cause of death, autopsy should reveal edematous lungs weighing in excess of 500 g along with a full bladder. Without these findings, a conclusion that the person died from an overdose of depressant drugs should be suspect.

### Drug Polymorphisms and Pharmacogenetics (Pharmacogenomics)

Polymorphism means difference in phenotype between individuals, and pharmacogenetics is the study of the genetic variations that cause differences in the drug response among individuals. These terms are used interchangeably, along with descriptions of “fast” and “slow” metabolizers. One of the first isozymes to be characterized was N-acetyltransferase 2, which causes the acetylation of isoniazid. Other drugs that have been shown to have variable metabolism as a result altered genetic makeup include succinylcholine (defects in pseudocholinesterase), nortriptiline and codeine (CYP2D6), mephenytoin (CYP2C19), warfarin, phenytoin, tolbutamide (CYP2C9*3), midazolam (CYP3A5), nicotine (CYP2A61B), methadone, selegiline, propofol, efavirenz, and cyclophosphamide (CYP2B6).

### SUMMARY

This article is intended to provide the reader an overview of principles, procedures, and practices in a modern forensic toxicology laboratory. Future trends in forensic toxicology may include a change in analytical procedures where LC tandem mass spectrometry, time of flight mass spectrometry, and/or capillary electrophoresis are the standard analytical methods. Certification of analyst and accreditation of laboratories will be a universal requirement. Laboratories will be able to minimize the use of paper by using electronic documentation. The overall effect of becoming a paperless laboratory will be an increase in efficiency, decrease in case turnaround times, and a reduction in cost. Interpretation of toxicology results from the perspective of pharmacogenetics will undoubtedly affect decisions concerning the cause and manner of death, as well as in legal proceedings. For further reading, an excellent introductory text, *Principles of Forensic Toxicology* has been provided by Levine.
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