

Chapter 66

Forensic Toxicology

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When the term toxicology is used, one thinks of poisons or exposure to something that will cause harm to the body. Indeed, toxicology does embrace the study of deleterious effects of substance exposure not only to the human body but also to the environment and all other organisms existing in the environment. Forensic toxicology represents a subset of toxicology where legal issues require toxicology studies to determine the facts. Forensic toxicology has been referred to as toxicology with medicolegal applications. The forensic toxicologist is a scientist with basic training and education most often in chemistry, pathophysiology, and pharmacology and frequently holding an earned graduate degree. Many forensic toxicologists have a PhD degree in pharmacology, toxicology, chemistry, or a related science. Forensic toxicology studies are nearly always analytical in nature because the interpretation of the actions and effects of drugs and toxic substances requires knowing what is present and how much is present. This is true regardless of whether the questions involve postmortem, human performance, or drug-testing interpretations. These are the areas in which forensic toxicologists are routinely involved: postmortem forensic toxicology, human performance toxicology, and forensic drug testing.

POSTMORTEM FORENSIC TOXICOLOGY

Postmortem forensic toxicology involves analyzing body fluids and organs from death cases and interpreting that information. Sudden unexpected and/or unexplained deaths become coroner's cases or fall under the jurisdiction of the medical examiner. Frequently in these cases toxicology studies are useful and necessary for the final decision regarding the cause and manner of death. In nearly every death that remains unexplained after postmortem examination, toxicology studies are sought to rule out poisoning, drug overdose, or therapeutic misadventure.

In some cases there is a history and/or physical evidence to indicate an overdose or poisoning, such as intravenous drug use and drug paraphernalia at the death scene, presence of suicide notes, or empty drug containers. A death from

an accidental fire or arson, or exposure to incomplete combustion fumes (motor vehicle exhaust), will indicate that carbon monoxide poisoning should be suspected. In these instances, forensic toxicology studies are necessary to corroborate investigative findings. However, the young or middle-aged therapeutic drug user found dead, the nursing home patient found dead, the science researcher found dead, all without a history or any physical evidence of poisoning or overdose, can present a problem that may be solved by toxicology testing and interpretation.

Forensic toxicologists routinely test postmortem blood and urine specimens when available. Various other fluids, e.g., eye fluid, stomach contents, and bile, also can be analyzed. Samples of organ tissues may have to be tested when bodies are decomposed and fluids are not available. Some forensic laboratories will test both a heart blood sample and a peripheral (femoral) blood sample in order to evaluate postmortem changes in blood concentrations. It is important for toxicology specimens to be properly collected during the autopsy process. Care must be taken that specimens are not contaminated with fluids from other compartments of the body. It is also recommended that a portion of blood specimens be preserved with fluoride to minimize post-mortem degradation.

Analytical methodologies used by forensic laboratories vary, but most use a combination of immunoassay and chromatographic methods to identify and quantify drugs and poisons. Alcohol is routinely analyzed in forensic laboratories by gas chromatography. Enzymatic and colorimetric methods occasionally are used as an initial or screening test. Carbon monoxide testing can be performed by spectrophotometric differentiation between oxyhemoglobin, reduced hemoglobin, methemoglobin, and carboxyhemoglobin. Carbon monoxide analysis is also done by a diffusion and colorimetric method, and gas chromatography. Cyanide testing is done by diffusion and colorimetric quantitation. Immunoassay testing can be used for screening both blood and urine specimens for a variety of drugs and drug classes. Opiates, amphetamines, barbiturates, benzodiazepines, and cocaine metabolite are examples of immunoassay testing. Chromatographic methods such as thin layer chromatography (TLC), gas chromatography

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(GC), high performance liquid chromatography (HPLC), and chromatography interfaced with mass spectrometry (GC/MS, GC/MS/MS, LC/MS, LC/MS/MS) are used for qualitative analysis and quantitative testing of specimens for drugs and poisons. For heavy metal poisoning such as arsenic, mercury, cadmium, and lead, specimens can be analyzed by atomic absorption spectrophotometry.

For the results of toxicology testing to be scientifically valid, the methods and procedures used for analyzing specimens must be validated. The validation process ensures the accuracy, precision, and specificity of the method. The process includes identifying limits of detection and lower and upper limits of quantitation. Included in method validation is testing for possible interfering substances and evaluating carryover from previous tested samples. The method must be able to provide accurate results for reference specimens. The forensic toxicologist must understand the importance of validation and be able to evaluate the effectiveness of the process. Results from scientifically valid methods are necessary to support medicolegal circumstances of criminal or civil cases.

A number of references are available for comparing blood concentrations in order to interpret the results of toxicology testing. Most, if not all, of the reference values for toxic and lethal concentrations of drugs and poisons appearing in the literature are from case reports. For reported reference values a wide diversity exists for methodologies used, condition of the specimens, and the validity of the testing in these reports. This leads to a wide range and frequently overlapping ranges of concentrations reported for toxic and lethal concentrations of drugs and poisons. Accurate and traceable analytical reference values for most scientific comparisons require experimental doses of substances under controlled conditions with validated procedures. Obviously this type of information is not available for toxic and lethal doses in humans. However, for many drugs valid and traceable reference values are available for therapeutic doses. The postmortem forensic toxicologist therefore must make interpretations based on data that in some cases are not scientifically sound. Information and data from case reports, however, is better than having no information available. This emphasizes the importance of experience and training necessary for interpreting postmortem concentrations of drugs and poisons.

Interpretation of combined drug toxicity can be particularly challenging. If the combination includes several or more drugs with similar mechanisms, such as central nervous system depressant action, it may be somewhat easy to interpret. However, when the combination includes drugs with different mechanisms or antagonistic mechanisms, such as selective serotonin reuptake inhibitor, central nervous system stimulant, and central nervous system depressant, the interpretation can be more difficult. This is an area where case reports involving combined drug toxicities can be helpful for interpretation and also the experience and training of the forensic toxicologist.

Blood samples from fatalities of motor vehicle accidents are routinely tested by postmortem forensic toxicologists for alcohol and also frequently for drugs. Alcohol (ethanol)

and many different drugs can render a person incapable of safe driving. This is clearly shown by the yearly statistics issued by the National Traffic Safety Bureau on motor vehicle deaths involving alcohol and drugs. A forensic toxicologist's role in interpreting impairment from alcohol and/or drugs is emphasized in human performance toxicology.

HUMAN PERFORMANCE TOXICOLOGY

Human performance toxicology is an area of forensic toxicology that primarily deals with driving under the influence of drugs and alcohol. Human performance toxicology can also be referred to as behavioral toxicology dealing with an inability to perform in the workplace.

The forensic toxicologist is frequently asked to interpret blood alcohol concentration (BAC) and blood drug concentration and the relationship they have with impairment. In addition, since the tested value for alcohol or a drug is not done on a sample taken at the time of the incident (accident or arrest) but at a later time, it is often necessary for the toxicologist to extrapolate what the person's BAC or drug level was at the time of arrest or the accident. This type of interpretation of blood alcohol and drug concentration and the effect on an individual relies on an understanding and knowledge of the physiology and the pharmacology of ethyl alcohol and drugs.

ALCOHOL IN THE BODY AND ITS EFFECTS

Ethyl alcohol, or ethanol, is the active constituent (drug) that is contained in alcoholic beverages. Ethanol concentration in beverages varies from a low of 4% to 5% in beers, 7% to 12% in wines, 20% to 40% in cordials, to a high of 40% to 50% in most distilled beverages (whiskeys, vodkas, rums, etc.). Proof strength stated on the labels of some beverages is a value that is double the percent strength. An example is that a 100 proof beverage would be 50% alcohol. It is necessary for the forensic toxicologist to know the concentration of ethanol in beverages in order to interpret quantities consumed related to blood alcohol concentration.

A person drinking an alcoholic beverage will not absorb alcohol into the blood while the beverage is in the stomach. The stomach's function does not include absorption but merely prepares and liquefies swallowed contents for emptying into the small intestine. When the beverage passes from the stomach to the small intestine (duodenum), absorption into the blood will occur. This process takes approximately 20 to 30 minutes for complete absorption when the stomach is empty. When food is present in the stomach the process takes longer due to food causing the beverage to stay in the stomach longer, thus extending the time of absorption. Depending on the amount of food present in the stomach, the time for complete absorption of alcohol may take an hour or longer.

Once alcohol is absorbed into the blood, it will be distributed to all parts of the body. In the brain, alcohol

has its primary pharmacological effect by producing central nervous system (CNS) depression. All of the impairment effects of alcohol are related to the depressant actions on the nervous system. These effects include increased reaction time, decreased visual acuity, decreased peripheral vision, poor judgment, and sensory-motor incoordination. The combined effects are referred to as "impairment" or "under the influence." Scientific studies have shown that impairment from alcohol can be related to the concentration of alcohol in the blood. Various concentrations are used by states and included in their statutes and regulations governing licensed drivers. Most states use a concentration of 0.10% or greater in blood to indicate that a driver is impaired. However, allocation of federal highway funding is being used to influence states to lower the concentration to 0.08%.

Once alcohol enters the blood and is distributed to the liver, it is metabolized first to acetaldehyde and then to acetate, providing calories. Approximately 90% to 98% of alcohol is metabolized, which occurs at a constant rate (zero order). A person's rate of metabolism depends on their experience and frequency of alcohol use. Heavy drinkers will metabolize more rapidly than light or nondrinkers. Metabolism plus the amount of alcohol excreted unchanged represents elimination or dissipation rate. Elimination rates average from 0.015% per hour to 0.02% per hour.

A person's blood alcohol concentration (BAC) can be estimated using the following formulae:

$$150/BW \times A/50 \times B \times 0.025\% = \text{Maximum BAC}$$

$$\text{Dissipation} = \text{Number of hours consuming beverage} \times \text{Elimination rate}$$

$$\text{Maximum BAC} - \text{Dissipation} = \text{BAC}$$

where BW = body weight, A = percent concentration of alcoholic beverage, and B = number of ounces of alcoholic beverage. It is necessary to know the person's body weight, the amount of beverage, and the percent alcohol content in the beverage. Also it is necessary to know when the person started drinking and when they finished to make a meaningful estimation.

Rates of dissipation and absorption of alcohol are also used by the forensic toxicologist to extrapolate back to the time of the arrest or accident. An example is an accident occurring at 8:00 P.M. There is suspicion of driving under the influence of alcohol (DUI). The driver does not pass a field sobriety evaluation and is administered a breath test 2 hours after the accident at 10:00 P.M. The result of the breath test is 0.095% BAC. The driver reports that he has not had anything to eat since lunch and that he stopped drinking 2 hours earlier at 6:00 P.M. Using the information provided and the range of average elimination rates, the driver's BAC at the time of the accident can be estimated. The driver's BAC would have been between 0.125% and 0.135% BAC. These values were obtained by adding to the BAC at 10:00 P.M. the dissipation that occurred over 2 hours. Calculations become more complex when drivers have eaten meals recently and continued to drink right up to the accident or arrest. These types of cases present a challenge for the forensic toxicologist.

DRUGS AND DRIVING

For blood drug concentrations and impairment, scientific studies similar to alcohol studies do not exist except for marijuana. Interpreting blood drug concentrations and impairment while driving is more difficult than interpreting impairment with alcohol. Some states utilize trained police officers as drug recognition experts (DREs). The DRE evaluates a suspect by administering a series of tests that are more comprehensive than a field sobriety test, as well as a breath test. In addition, measurements of pulse, blood pressure, and body temperature are taken. After evaluation of all test information the DRE forms an opinion as to what drug or drug class is causing impairment. A blood or urine sample is also taken for toxicology testing to support the DRE's decision. Testing only a urine specimen provides for evidence of prior exposure but cannot provide a direct relationship to impairment. Opinions of forensic toxicology experts regarding impairment from drugs generally rely on blood concentrations and not on urine concentrations.

FORENSIC DRUG TESTING

In 1986, President Ronald Reagan issued Executive Order No. 12564, indicating that the federal government would be a drug-free workplace. Earlier in 1983, a study by the National Transportation Safety Board involving drugs and alcohol use in railway accidents prompted the Federal Railway Administration and the National Institute on Drug Abuse (NIDA) to begin developing drug regulations. Initially the intent was to have guidelines for the Department of Transportation (DOT), but with Reagan's executive order, NIDA continued to investigate the appropriateness of drug testing through studies and conferences. In 1988, NIDA issued mandatory guidelines for federal drug-testing programs. The guidelines were comprehensive, including issues of confidentiality, choice of specimen and collection, chain of custody, procedures for testing, quality control, records, reporting results, and interpretation of results. The regulation guidelines provided for a medical review officer (MRO) to review results before final reporting. Also included were guidelines regarding accrediting laboratories, inspecting laboratories, and proficiency testing for laboratories to maintain accredited status. A National Laboratory Certification Program administered by Research Triangle Institute commenced in 1988 under the auspices of the Department of Health and Human Services through NIDA. Drug testing under the guidelines is applicable only to federal employees and federal agencies, but private-sector drug testing quickly adopted many of the "NIDA guidelines" for their programs.

Although forensic drug testing had been utilized much earlier in the military and Olympics, and also in a small segment of private industry, before 1988, it was Reagan's executive order and subsequent NIDA guidelines that caused a tremendous growth in forensic testing. All types of industry, both large Fortune 500 and smaller companies, began to develop drug-free workplace policies that included drug testing. In addition, many other organizations

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or specific populations instituted, modified, or increased their use of forensic drug testing to achieve certain objectives. Testing of athletes, both professional and Olympic as well as high school athletes, insurance testing, drug rehabilitation, probation, and parole monitoring are examples of forensic drug testing. Drug testing in hospitals or clinical reference laboratories generally is done for medical purposes, but may become forensic testing when involving legal questions.

Federal regulations specify urine as the specimen for testing. Federal regulations allowed testing urine for five drugs or drug classes: cannabinoids (marijuana metabolites), cocaine (benzoylecgonine), amphetamines (amphetamine and methamphetamine), opiates (morphine and codeine), and phencyclidine (PCP). Nonfederal urine drug testing has been expanded to include barbiturates, benzodiazepines, methadone, LSD, and propoxyphene. Two separate portions (aliquots) of a urine specimen are required to be analyzed for a test to be reported as positive. An initial test on the first aliquot must be an immunoassay method. If the initial test is positive, a confirmatory test that must be a gas chromatograph/mass spectrometry (GC/MS) method is done on a second aliquot. Both the initial immunoassay test and the GC/MS confirmatory test are required to be validated by the laboratory. Validation criteria are discussed above in the postmortem forensic toxicology section.

For each test, the regulations provide cutoff concentrations to indicate a presumptive positive and a confirmed positive result. A specimen aliquot that tests negative on the initial test is reported as a negative and does not require any additional drug testing. The cutoff concentrations were chosen based on experience and recommendations by toxicologists involved with military, postmortem, and clinical testing for drugs. The initial test cutoff values for cannabinoids and opiates were found to be inappropriate and have since been changed. The original urine initial test cutoff for marijuana metabolites was 100 ng/ml. It was shown that this concentration was too high and likely resulted in many false negative reports. Many products promoted to "beat the drug test" that were based on drinking additional water were thought to be effective merely due to dilution of the urine. Subsequently the cutoff for marijuana metabolites was lowered to 50 ng/ml. This value was effective in decreasing the number of false negatives and is high enough to avoid a positive test due to passive inhalation.

The original cutoff for opiates was 300 ng/ml. Foods containing poppy seeds were found to produce positive urine results for morphine. Poppy seeds contain enough morphine to produce urinary concentrations above 300 ng/ml. In addition, cough medicines containing codeine produced urinary concentrations greater than the cutoff for a positive. Prescription analgesics taken by many individuals contain codeine along with a nonopiate analgesic. Chronic use of these prescription analgesics can produce morphine in the urine as a metabolite of codeine. To avoid positives for unintentional exposure and medicinal therapy, the initial test for opiates and confirmatory test cutoff concentrations for morphine and codeine have been changed to 2000 ng/ml for opiates.

Heroin use may be related to a positive result for morphine in the urine. Since heroin is diacetylmorphine and morphine is a heroin metabolite, morphine in the urine can indicate prior use of heroin. Since morphine can be present in the urine from sources other than heroin, the federal regulations allow for a definitive test for heroin use. Urines positive for morphine can be tested for monoacetyl morphine, a metabolite that can only be produced in the urine from heroin use.

Nearly all workplace urine drug testing is done with unobserved collections. This has led to some problems because of numerous attempts to thwart the testing process. The collection process includes monitoring the temperature of freshly collected urine. If there is an attempt to substitute another urine it is difficult to maintain the correct temperature. Various products have been available for sale that can be added to the urine to interfere with testing. They are referred to as adulterants. Some adulterants are very effective and are difficult to detect. In addition, individuals who are intent on "beating the test" will drink large amounts of water or other fluids prior to a test. This will dilute the urine and can cause a false negative when the concentration in the urine is less than the cutoff concentration.

Federal guidelines allow certified forensic laboratories to test for dilution, substitution, and adulteration of urine samples. There are guidelines established for dilution of urine based on creatinine and specific gravity, and for pH regarding adulteration. Most laboratories will test urines for creatinine content and pH. Creatinine concentration in the urine and specific gravity can provide a measure of dilution. Adulteration of urine is frequently done by addition of an oxidizing substance such as a nitrite salt or pyridinium chromate. These issues present a challenge for the forensic toxicologist.

A medical review officer (MRO) is required to review results of federally mandated drug tests. The MRO can interview and medically evaluate a person with a positive drug test. Based on their findings, the MRO can modify the reported result. An MRO can also request a retest of the specimen.

Specimens other than urine are also being tested for evidence of drug use. Hair is being utilized for testing by some private-sector companies. Oral fluid testing is also being used to detect illicit drug use. These alternative specimens are currently being evaluated as possible substitutes for urine drug testing.

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