Dead Men Do Tell Tales: Location, Recovery and Testing of Human Remains

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This curriculum unit is recommended for:
Honors Forensic Science and Advanced Forensic Science
Grades 11 and 12

Keywords: drug redistribution, postmortem drug levels, pharmacokinetics, forensic toxicology, forensic pathology, antemortem drug levels, locating unmarked graves, bioavailability, lethal dose, blood alcohol content, grave excavation

Teaching Standards: See Appendix 1 for teaching standards addressed in this unit.

Synopsis: This curriculum unit explores advanced material in forensic anthropology, pathology and toxicology. Detection of unmarked graves by various techniques is discussed followed by a practical exercise in grave excavation. The unit then turns the focus to determining postmortem drug levels in body tissues. The pharmacokinetics of drugs in the body is explored in detail. Once the processing of drugs in the body before death is understood, the unit moves to postmortem drug testing and the problems that postmortem redistribution of drugs in the body pose to determining antemortem drug levels. The concept of lethal dose is introduced and explored through lab work. Finally, the unit addresses how to calculate drug and alcohol levels in the body as well as the toxicity of substances in the body.

I plan to teach this unit during the coming year to 65 students in Advanced Forensics.

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Introduction

Forensic Science is no longer a game of "I Spy" with a magnifying glass. This area of science is building on developments and discoveries in the fields of chemistry, biology, physics and more to rapidly become one of the most technologically advanced sciences. The sheer number of television shows devoted to the field, never mind the entire channels on cable, attest to the public's fascination with forensics and solving crimes. Students are finding that they enjoy science in this context and many are considering careers in the sciences for the first time. Charlotte Mecklenburg Schools offers two forensic science courses for high school students as their fourth year science electives – Honors Forensic Science and Honors Advanced Forensic Science. The first level course exposes students to many popular areas of forensic science such as fingerprint analysis, DNA analysis and arson. The second level course builds on some of the basic material but extends it further and includes new topics such as Forensic Botany, Digital Forensics and Accident Reconstruction. The Advanced Anthropology and Advanced Pathology units are the focus of this curriculum unit.

Rationale

Charlotte Mecklenburg Schools' standards for Advanced Anthropology include students being able to use environmental clues to locate an unmarked grave and then use correct techniques to excavate the grave and recover the remains. Pathology standards state that students should understand how to recover and store trace evidence during an autopsy and how to extract blood and urine samples. Advanced Pathology requires students to explain how laboratory tests are used to determine factors that may have contributed to the death of a person, as well as determining what samples to take and what tests should be done during an autopsy. Toxicology standards state that students should understand the process of isolating and identifying drugs, toxins and poisons in human tissue and understand and appreciate the difficulties in isolating drugs, toxins and poisons in human tissue. The standards for Advanced Analytical Techniques provide for students to compare and contrast the many types of screening and confirmatory tests for drugs and to learn about the use of advanced technology such as the use of ground penetrating radar and decomposition odor detectors in the search for unmarked graves.

Before a medical examiner can conduct an autopsy to determine cause and manner of death, investigators must locate remains that may have been left in unmarked graves for some time. Forensic anthropologists are now able to use ground-penetrating radar to locate anomalies beneath the surface to help focus investigators' searches. A new

technology called LABRADOR - short for Light-Weight Analyzer for Buried Remains and Decomposition Odor Recognition - is being used successfully for the same purpose.

Once remains have been recovered, an autopsy takes place if there is soft tissue remaining. Students will become familiar with the procedures performed during a forensic autopsy including what types of tissue samples to take for testing and how to obtain, package and preserve those samples. Students will study the various types of tests that are performed on the samples taken and how the results help determine factors that may have contributed to the death of the person.

Students will learn about the pharmacokinetics and the pharmacodynamics of drugs of abuse including alcohol, cocaine, heroin, methamphetamine, barbiturates and others. Pharmacokinetics is the way a drug moves through the body. Pharmacodynamics involves the effects of the drug on the various body systems as well as its mechanism of action in the body. Students will also come to realize that the levels of drugs and their metabolites in the body can change very rapidly after death.[1]

Student will conduct a drug analysis lab which will give them exposure to various screening and confirmatory tests for the presence of various drugs. They will conduct color tests and work with common over-the-counter medications as well as simulated marijuana and simulated LSD.

Once substances in the body have been identified and quantified, the investigator must determine if the findings are relevant to the cause of death of the person, that is, whether the concentration of drugs in the body rose to the level of toxicity required to cause death. Students will learn about LD50 which stands for Lethal Dose 50%. It is the concentration of a substance at which 50% of exposed organisms will die.[2] Students will work with LD50 tables and simulated data sets to explore the toxicity of various drugs of abuse. Students will also conduct a lab to determine the LD50 of various household chemicals on brine shrimp.

Demographics

William Amos Hough High School is a large suburban high school of over 2700 students located in the small town of Cornelius, North Carolina just north of Charlotte. We opened our doors in 2010 to serve the northern part of the Charlotte-Mecklenburg School District. Eighty-four percent of our graduates go on to either two- or four-year colleges while 16% join the military. Twenty-six present of our students are minorities and 18% are free and reduced lunch students. We offer a comprehensive college preparatory program in the arts and sciences. Classes are taught at the Standard and Honors levels and we offer 26 Advanced Placement courses in conjunction with the College Board. Our science program requires one earth science (Earth and Environmental Science or AP

Environmental Science), Biology, and one physical science (Chemistry, Physics or Physical Science). Biology, Chemistry and Physics are also offered at the AP level.

Students are required to take either a fourth year science or social studies course. We offer Honors Forensic Science and Honors Advanced Forensic Science to meet that requirement. With the overwhelming popularity of forensics in pop culture, these courses grab students' interest while teaching them valuable lab skills and critical thinking.

Content Background

Locating Remains

Before postmortem testing can be done, investigators must have a body to work with. It is unfortunately too frequent that people go missing and searches are mounted to locate them. Large areas of land need to be searched. Sometimes killers dispose of their victims' bodies by burial in unmarked locations. Since one of the goals of this curriculum unit is to expose students to advanced technologies in forensic science, it makes sense to start with ways to detect clandestine burial sites. Ground penetrating radar and odor detection devices are two of the ways this can be done.

Ground penetrating radar (GPR) is exactly what it sounds like. It works by sending electromagnetic waves into the ground.[3] If the waves strike a solid substance, such as a buried body, the waves will reflect back to the surface. The time it takes for the waves to reach the surface is correlated to the depth of the object. The success of GPR depends on factors such as the composition of the soil and surrounding objects. GPR works best in sandy soil where the waves can pass more freely than in hard clay soil. Bodies that are wrapped in a blanket or something tend to reflect the waves back better than naked bodies. GPR also has the advantage of being able to identify other buried objects such as crime weapons. GPR is used indoors as well to help locate bodies buried under floors or in walls.

Cadaver dogs have been used for some years now to locate buried remains not just in homicide situations, but in cases of building collapses and other mass disasters.[4] The canine sense of smell is so sensitive that it can detect the odor of a decaying body underground. We know that blowflies arrive at dead bodies to lay eggs within minutes of death. There must be some odor released that alerts them to the body. Decomposition of remains causes the release of more than 400 different chemicals that have very distinctive odors. [5]Scientists at Oak Ridge National Laboratory in Tennessee have developed a hand-held analyzer that can let searchers know when buried human remains are nearby. Known by the acronym LABRADOR, for Lightweight Analyzer for Buried Remains and Decomposition Odor Recognition, the device detects the major classes of volatile chemicals present during decomposition.[6] It can be more accurate than canines or GPR as it identifies the unique chemical signature associated with human decomposition. It can

also help determine the time of death based on the strength of chemicals remaining. Since decomposition can be affected by humidity and weather, the signature odors released during decomposition can vary widely and more work is being done to standardize the field.

Recovering Remains

Once an unmarked grave has been located, it is necessary to exhume the remains for identification and possibly other forensic purposes. To conduct a field exhumation, certain minimal equipment is required.[7] A T-shaped probe is used to pierce soil looking for differences in soil density in different locations. Disturbed soil, or soil that has been dug up and replaced, will be less dense than surrounding undisturbed soil. Once the outline of the actual grave is ascertained, workers should gather rakes, square-tipped shovels, hand trowels and brushes as well as colanders or sifting devices. The entire area should first be searched for potential evidence that may be lost in the chaos of an excavation. The area should be thoroughly photographed, mapped and documented as well.

Once the team is ready to begin, roles need to be assigned. Someone must be the recorder. Their job is to maintain a written record of everything that goes on at the excavation site. This includes lists of all workers and visitors. It also includes keeping an excavation log, which tracks what workers are doing and the sequence of recoveries, as well as an evidence log which assigns a unique identifier to each piece of evidence or remains recovered. The material is then properly packaged and prepared for transport.

The mapper will create and update 2D and 3D maps of the excavation site. They must measure the site before excavation begins and separate it into grids if it is large enough. They must record all natural and man-made features on their maps. They should also include GPS coordinates for future reference. They also maintain a record of every feature or piece of evidence found. Work must stop when something is discovered so that the mapper can properly document the find.

The photographer keeps a photographic record of the entire project from arrival to after the site is returned to its natural condition. They must photograph all of the evidence and remains found. They will also photograph the ongoing work so that there is a complete record of the entire project. They are responsible for maintaining a photo log which contains the date, time and subject of each photo. It may be helpful to have a second person keeping the log while the photographer snaps the pictures.

The rest of the team is made up of diggers. They will follow a set process to excavate the grave. First, they will remove any litter and vegetation over the grave site, being careful not to destroy anything that could be evidence. Second, they will use flat shovels to carefully lift horizontal layers of dirt off the grave site until the topsoil is removed and

the outline of the grave is revealed. The grave will then be measured, photographed and mapped. Third, they will continue to remove horizontal layers of dirt until they notice a change in the soil density, color or texture at which point they will switch to using hand trowels. Sifters will be running all of the soil removed from the grave through strainers or colanders to look for pieces of evidence. Next, if they are able, the diggers will "pedestal" the remains.[8] This means digging down around the outside of the remains so that there is a trench around the body. This allows more control when recovering the remains and results in less damage to the remains. Fifth, workers will use brushes to clear off each individual piece of evidence and free it from the ground. Each piece of evidence or remains must be tagged with a unique identifier and appropriately packaged. Lastly, the digging will continue until "sterile" soil, or soil which has not been disturbed and which is beneath the level of the grave, is reached. The site must then be cleaned up, holes refilled and final documentation of the site made before returning to the medical examiner's office with the remains for identification and testing.

How Drugs Work in the Body

Now that a body has been brought to the medical examiner's office for investigation, many types of forensic testing will come into play, particularly in cases where drugs are suspected to have contributed to the death. In order to understand how drugs in the body can contribute to the cause of death, it is first necessary to understand how drugs work in the body. This area of study encompasses two parts: pharmacokinetics and pharmacodynamics. Pharmacokinetics deals with the movement of drugs in the body including how drugs are absorbed into the body, how they are distributed throughout the body, how they are metabolized in the body and how they are finally excreted from the body.[9] Pharmacodynamics deals with the effects of the drugs at their target sites and the mechanisms of their actions.[10]

Absorption

Absorption is the process whereby xenobiotics[11] enter the bloodstream.[12] The two main mechanisms by which drugs cross into the bloodstream are passive diffusion and facilitated diffusion.[13] In passive diffusion, the drug is moving from an area of high concentration to an area of lower concentration. For example, when a drug is injected into muscle tissue, it will diffuse into nearby blood for transport throughout the body. Certain membrane proteins may help the drug diffuse along a concentration gradient which is known as facilitated diffusion. Neither of these processes require energy. Active transport requires energy for carrier proteins to move the drug across barriers into the bloodstream. The use of carrier proteins results in very large molecules which decrease the efficiency of diffusion. In addition, there may be a finite number of carrier proteins which will limit the amount of drug that can cross into the bloodstream.[14]

There are many routes of drug administration. Various drugs are more effective with different methods of administration because of the way they absorb into the body. The table below shows some methods of administration and how they work in the body.

Table 1. Common methods of drug administration to the body[15]

Method of Administration	**How They Work in the Body**
Oral	- Most common - Absorption occurs in gastrointestinal tract (stomach and small intestine)
Inhalation	- Volatile drugs can be absorbed through the lungs
Intravenous	- Most efficient because drug goes directly to bloodstream
Intramuscular	- Absorption between different people can vary
Rectal	- Drug is absorbed by the tissues of the colon - Useful when drugs cannot be taken orally, such as anti-nausea medications
Sublingual	- Drugs administered under the tongue absorb very rapidly - Common example is nitroglycerin for chest pain
Transdermal	- Drugs are absorbed through the skin such as nicotine patches
Ocular	- Usually drops for eye ailments delivered directly to the eye
Intranasal (Insufflation)	- Drugs that are absorbed into the body by the mucous membranes in the nose such as cocaine

Bioavailability

Bioavailability is the amount of a drug absorbed into the body relative to the amount of drug administered.[16] It is assumed that 100% of a drug delivered by intravenous injection is immediately absorbed into the bloodstream. There are factors that affect the bioavailability of a drug under different circumstances.

Drugs must be in solution in order to enter the bloodstream. Drugs that are taken orally in tablet form need time to disintegrate and enter solution which slows the bioavailability of the drug. Extended release or coated tablets take even longer to dissolve.

The amount of blood supply can change the bioavailability of a drug in the body. When blood flow speeds up, absorption of a drug can increase. On the other hand, if blood flow slows, due to shock, for example, then absorption of the drug can also slow.

The stomach is acidic (pH 1-3.5)[17]. Acidic drugs will be mostly not ionized in the stomach and therefore more easily absorbed at that location. The pH of the duodenum is 5-6 and the ileum is 8. The pH rises as the drug moves through the digestive system. This means that as an acidic drug moves on, the amount of drug in the un-ionized form decreases which means absorption decreases. The opposite is true for basic drugs. They are more readily absorbed in the intestines than in the stomach.

Distribution

Distribution is the movement of the drug from one part of the body to another.[18] The drug has been administered and absorbed into the bloodstream by some mechanism. The drug now needs to reach the target tissues so it can do its job. Tissues in the body that have a great deal of blood flow through them such as the liver, heart and kidneys will get the bulk of the drug first. Other tissues such as muscle and fat will take longer to build up high concentrations of the drug.[19]

There are several factors that can influence drug distribution in the body. The more lipid-soluble the drug, the more easily it will move into the tissues.[20] If the drug tends to bind to plasma proteins, it is not free to leave the blood and move into the tissues. It is important to know how widely a drug will distribute in the body because this will affect plasma concentration and the size of the dose to be administered. This can be measured by the volume of distribution (V_d). The volume of distribution of a drug is a measure of the degree to which a drug is distributed in body tissues rather than in blood plasma.[21] The amount of drug introduced into the body (X) divided by the concentration of the drug in plasma (C_p) gives you the volume of distribution, or an idea of the size of the area over which the drug dispersed in the body.

$$V_d = \frac{X}{C_p}$$

This is so important because this concept is used to figure out concentrations of the drug in the body over time. For example, the V_d of acetaminophen is 0.9 L/kg. If a 70 kg person took an 800 mg dose, their expected plasma concentration (C_p) of acetaminophen would be 800 mg / (0.9 L/kg x 70 kg) = 800 mg / 630 L = 1.27 mg/L.

In a car crash fatality case being able to extrapolate blood alcohol concentrations prior to the time of the accident is essential to determining if alcohol played a role in the death. The Widmark Formula for calculating Blood Alcohol Concentration (BAC) is:

BAC = [Alcohol consumed in grams / (body weight in grams x r)] x 100
where r is a gender constant (0.68 for males, 0.55 for females)[22]

For example, a 70 kg man drank 4 beers over the course of 2 hours and then drove home and crashed into a school bus. He was taken to the hospital where a blood sample was taken. The police will want to know the driver's BAC at the time of the accident. A standard beer is approximately 5% alcohol by volume. A standard drink contains about 14 g of alcohol. The man consumed 4 beers containing 14 g of alcohol each for a total of 56 g of alcohol. His weight in grams is 70 kg x 1000 g/kg which equals 70,000 g. This gives the formula:

BAC = [56 g / (70,000 g x 0.68)] x 100
= [56 g / 47,600] x 100
= 0.001176 x 100
= 0.12

Now the police will factor in the about of time it took to consume the drinks. No one would argue that four drinks over the course of 24 hours would result in impaired driving. However, in this case, the four beers were consumed in two hours.

BAC at time of driving = BAC – (elapsed time in hours x 0.015)
= 0.12 – (2 x 0.015)
= 0.12 - 0.03
= 0.09

The 0.015 is the rate of elimination of alcohol from the body.[23] Even though the alcohol was spread out over a couple of hours, the driver still would have had a BAC over the legal limit to drive at the time he got behind the wheel.

Metabolism

Metabolism is the process by which the structure of a xenobiotic is altered to products called metabolites in order to facilitate its removal from the body.[24] Metabolism is broken into two phases. Some drugs undergo either Phase 1 or Phase 2 metabolism, but most undergo Phase 1 metabolism followed by Phase 2 metabolism.[25] During Phase I, the drug is being oxidized, reduced, hydrolyzed or otherwise chemically altered to facilitate its use at its target site and its ultimate elimination from the body. These reactions are characterized by the enzymatic transformation of functional groups.[26] The most common enzyme used is Cytochrome P450. Enzymatic activity takes place mainly in the liver.

Phase II metabolism involves conjugating, or joining, the metabolite with another polar molecule in order to increase the water solubility of the metabolite.[27] This makes the new molecule more easily filtered by the kidneys.

When drugs are administered orally, it is sometimes possible for the enzymes in the digestive tract to metabolize the drug before it reaches the bloodstream for transport to its site of action. This is known as the first-pass effect.[28] Drugs which exhibit a significant first-pass effect cannot be administered orally. This is also the reason that many illicit drugs such as cocaine and heroin are smoked or injected rather than ingested. Both drugs have a strong first-pass effect when taken orally so that very little of the drug – and hence its effects – acts on the body. Inhalation by smoking or snorting and injection get the drug very quickly into the bloodstream for distribution throughout the body. This results in a much quicker and stronger effect.

Excretion

Excretion is the removal from the body of the xenobiotic and any byproducts connected with it.[29] Drugs can be excreted in sweat and in breast milk. They can be exhaled through the lungs. The most common forms of excretion however are hepatic and renal.

The liver is the main site for metabolism of drugs. The liver creates bile from waste products which is stored in the gallbladder. It eventually enters the intestines and is ultimately eliminated as feces. The amount of blood flow to the liver can affect the clearing of drugs from the bloodstream. When blood flow is slowed, less drug can be removed.

The kidneys filter blood and remove waste. They are the primary organs of excretion. The material excreted through the kidneys is the waste that is filtered by the nephrons in the kidneys minus any drug that is reabsorbed from the kidneys into the bloodstream plus any drug secreted at the kidney.[30] Both metabolized and unmetabolized drug will be filtered by the kidneys. Reabsorption happens in the kidneys and can be a good thing. The body wants to reabsorb salts, sugars and other materials necessary for proper function. It would not be a good thing for drugs to be reabsorbed since there would be no way to control dosing if that occurred. The kidneys preferentially reabsorb lipid-soluble materials. Since the process of metabolism increases the polarity, or water-solubility, of the waste, drugs tend not to be reabsorbed in large amounts. Some drug that failed to be filtered by the kidney can still be secreted unto the urine before it leaves the body.

All of this becomes relevant to forensic science during death investigations where drug use is suspected. The questions become whether drugs were involved, what and how much drug was taken, when was the drug taken and did it cause the death. To answer these questions, investigators begin with the autopsy.

Autopsy

Now that the body has been recovered, and assuming that there is still soft tissue present, an autopsy needs to be performed to determine the cause, manner and mechanism of death. If the body has been identified, the forensic pathologist will begin by gathering all of the available information about the subject, including medical records and police and witness reports concerning the circumstances surrounding the death.[31]

Pathologists begin an autopsy with a thorough examination of the exterior of the body. They are looking for trace evidence such as gun powder residue, skin cells under the nails or any other thing that could contribute to determining the cause and manner of death. They will also look for and document any physical anomalies such as tattoos and birthmarks that can be used for identification. They will usually x-ray the body to look for signs of internal trauma or to located bullets in shooting cases.

Once the external exam is complete, the forensic pathologist will begin the internal examination. This starts with the familiar "Y" incision from both shoulder joints to the middle of the sternum and then down to the pubic bone. The rib cage is removed and the organs are examined in place. There are different techniques that pathologists can use at this point, but ultimately they all involve removing, washing and examining the internal organs. After being weighed and examined, the organs are placed in plastic bags and returned to the body cavity. The brain is frequently examined as well. This involves cutting a flap of skin on the head and folding it forward so that a saw can be used to remove the skull cap. The brain is removed, weighed and examined. Samples of brain tissue are taken and preserved and can be sent for toxicological testing.

During the internal exam, the forensic pathologist will take samples of tissue from various places in the body for testing. Each sample must be separately packaged, labeled and sealed and chain of custody documents must be started. Depending on the type of death being investigated, different samples may be taken. The most common are blood, urine, vitreous humor, gastric contents, bile, liver and hair.[32]

Blood is the primary source used for drug postmortem testing. Blood is taken from at least two sources: the heart (known as central blood) and the femoral artery (known as peripheral blood).[33] The different locations are critical to accurately determining the perimortem drug concentrations because these concentrations can change drastically after death depending on the type of drug and where in the body it is located.

The liver is the primary source of solid tissue for testing since most drugs are metabolized in the liver which means there may be significant levels of the drugs there. The vitreous humor in the eye is a good source for testing for drugs, particularly alcohol. It is isolated from the rest of the body and thus is less likely to be affected by postmortem drug distribution throughout the body. Stomach contents are helpful in suspected overdose cases when the route of administration is oral. There may be remnants of pills

or capsules in the stomach. The drug concentrations will likely be much higher in the stomach in the case of a quick death from an oral overdose.

Changes in Drug Levels in the Body After Death

It can be very difficult to extrapolate antemortem drug levels from postmortem samples. This is largely due to the fact that drugs in the body undergo postmortem redistribution. Postmortem redistribution is the sum of the processes that cause the movement of drugs and poisons between the blood, tissues, organs and other bodily fluids after death.[34] The table below lists some of the factors affecting drug concentrations after death.

Table 2. Factors Affecting Postmortem Drug Concentrations[35]

1	Circumstances of death
2	Time since death
3	Alteration of the body (ex. By embalming or putrefaction)
4	Position of the body during transport
5	Site of the sample collection
6	Method of collection
7	Preservation of the sample
8	Chemical stability of sample after collection
9	Postmortem metabolism
10	Bacterial metabolism of the drug
11	Postmortem drug synthesis
12	Drug redistribution

The body is considered to be divided into two compartments – the central compartment consisting of the heart, lungs, liver and other major organs and the peripheral compartment consisting of the muscles and fat tissue further out from the center of the body.[36] Many drugs deposit in the tissues of the central compartment. When a blood sample is taken from a central vein, the resulting drug concentration may be much higher than it was during life as the drug will have diffused back into the blood vessels near the major organs after death. On the other hand, redistribution into peripheral vessels is much less, which will result in a more accurate estimation of antemortem drug levels. Whenever possible, blood samples for toxicological testing should be taken from the femoral vein for this reason.

Postmortem Drug Testing

Both qualitative and quantitative tests for drugs are performed on postmortem samples taken during autopsy. A qualitative test is any test that indicates the presence of a drug without giving information on the quantity of drug present.[37] Immunoassays are common

screening tests that use antibodies to detect a reaction with specific drugs or their metabolites. There are drugs that are routinely tested for in these immunoassays including alcohol, analgesics, antidepressants, sedatives, cannabis, cocaine, opiates and stimulants. Other substances must be specifically tested for such as certain newer designer drugs like bath salts and spice, digoxin, GHB and LSD.

As a screening test, immunoassays are only able to detect whether a sample is positive or negative for a certain drug. The possible results from this type of testing are a positive (the drug is actually present), a false-positive (the test incorrectly states that the drug is present), a negative (the drug is not present) and a false-negative (the test incorrectly states that the drug is not present). All positive results from immunoassay screening tests must be confirmed with another type of test, usually some form of chromatography and spectroscopy.

A quantitative test involves determining the amount of drug present in the body.[38] The gold standard for this type of testing is chromatography coupled with mass spectrometry. Chromatography involves several techniques whose purposes are to separate compounds into their constituent parts. Forensic toxicology frequently uses high performance liquid chromatography (HPLC) or gas chromatography (GC). Both of these are combined with mass spectroscopy to identify and quantify the drug present in the sample.

Lethal Dose

Once substances in the body have been identified and quantified, the investigator must determine if the findings are relevant to the cause of death of the person, that is, whether the concentration of drugs in the body rose to the level of toxicity required to cause death. Most drugs have a therapeutic level much lower than their toxic or lethal levels. For example, while it is possible to fatally overdose on acetaminophen, the dose required to alleviate headache pain is far, far less than the amount required to induce a fatal overdose. There are some drugs where this difference is much smaller. Many chemotherapy drugs are highly toxic, and while they may be therapeutic for the cancer, their toxicity causes major side effects for the patient. For obvious reasons, scientific studies of the lethal dose of drugs cannot be conducted in humans. The evidence available is limited to drug concentrations found in postmortem samples taken from overdose victims. Extrapolating postmortem drug levels to antemortem drug consumption is an inexact process at best. This means there is very little reliable data on the lethal levels of most drugs in humans.

Toxicity is measured by the LD50 of a substance. LD50 stands for Lethal Dose 50% which is the dose at which 50% of the organisms exposed to the drug will die.[39] (See Appendix 2 for a table containing LD50 values for various substances.) The table below shows the different classifications of toxicity for materials that can be poisonous to humans along with the range of their LD50s.

Table 3. EPA Toxicity Categories for Human Poisons[40]

Category	Classification
I	Highly toxic and severely irritating
II	Moderately toxic and moderately irritating
III	Slightly toxic and slightly irritating
IV	Practically non-toxic and not an irritant

The toxicity of a material to a human of a certain mass can be estimated using LD50 data derived from studies on rats. The potential lethal dose of a material can be expressed as:

Lethal Dose in mg = LD50 in mg/kg of material x weight in kg

For example, to calculate how much nicotine it would take to kill a 70 kg man, you would multiply the LD50 of nicotine which is 50 mg/kg by the weight of the man which is 70 kg. The lethal dose of nicotine for this hypothetical man would be 3,500 mg. A human absorbs approximately 1 mg of nicotine from each cigarette smoked. So while it would be extremely difficult for this man to overdose on nicotine by smoking, the concentrated liquid form of the drug would be much easier to use to poison him.

Teaching Strategies

Teaching and learning strategies that will be highlighted in this unit include lab activities and small group and individual problem-solving work as well as reading, writing and reflection. Material will be introduced through brief lectures followed by hands-on activities. Students will work with problem sets covering blood alcohol concentration calculations, toxicity calculations and volume of distribution calculations. Students will conduct a lab based on LD50. They will be given access to various household chemicals and brine shrimp and will have to design and execute a procedure to determine the LD50 of a chemical. Students will also conduct a commercial lab on the analysis of drugs in order to try different drug testing techniques. Student will exercise their literary skills with a reading and writing project based on Deborah Blum's "The Poisoner's Handbook." Students will conduct small group discussions on various relevant topics as a mechanism for reflecting on their learning. Assessments for this unit will include the formal lab reports based on the two labs, problem sets based on simulated data, a book project and an end-of-unit test.

Classroom Lessons

This unit will take approximately 11 90-minute class periods to complete. This assumes the majority of "The Poisoner's Handbook" will be read by students at home.

Day 1

I will introduce the scope of this unit to my students by discussing concepts in anthropology, toxicology and pathology and the difficulty in testing postmortem samples for antemortem drug levels. “The Poisoner’s Handbook” by Deborah Blum is a very engaging history of the development of forensic medicine in New York City during the 1920’s. The whole book can be assigned for reading or the class can be split up into teams and assigned specific chapters. The chapters each cover different poisons so the class will explore a wide range of poisons by completing the whole book. Appendix 3 has links to various sites with activities related to the book. You can assign a formal project based on the book or just have students complete shorter activities based on the book.

In order the complete the first section of the unit, the teacher will need to obtain a skeleton or at least various bones in the human skeleton. I purchased a life-sized plastic skeleton on EBay for $150.00. Knowing I had approximately 60 students in my Advanced Anthropology course this year, I knew I needed to dig 6 graves for teams of 10 students each to excavate. I found an area free from casual traffic on the edge of campus and dug six 3’x3’ holes. In the holes I put 5 bones from the same area of the skeleton (simulating a serial killer who had dismembered the body and buried it in 6 different graves), two bullet casings, two pennies, and one water bottle/fast food drink cup. I filled in the holes and replaced, as best I could, the foliage from on top of the original area. I did this during the third week in August and I didn’t have my students excavate until the middle of October. This allowed enough time for the soil to settle and some of the foliage to regrow.

I will begin with brief notes on unmarked grave identification techniques and grave excavation procedures. Students may read and discuss an article such as “Three Advances in Forensics” or “LABRADOR: New Alpha Dog in Human Remains Detection?” for information on newer methods for detecting unmarked graves with decomposition odor detectors. Teams of approximately 10 students each will be assigned for grave searching and excavation. Students will reference the “Field Methods” chapter of the Forensic Anthropology Guide to develop detailed job descriptions for the project leader, records, mappers, photographers, diggers and sifters. The job descriptions should be specific enough that someone could read them and correctly perform that job. They will also develop a materials list which I will then provide for them. I will not give them anything they may not have thought of or forgotten to list! They will also create any forms they need for their jobs.

References for the articles and chapter are listed in the Bibliography. A list of materials to have on hand for this project is attached in Appendix 4.

Days 2 and 3

Let the students gather up the materials they requested and take the class outside to a spot somewhat away from the location of the graves. Allow teams to begin searching for unmarked graves. Once they think they have found one, they must call me over to confirm their find. This is so we don't dig up the entire campus! They will then properly mark, record, photograph and map their grave site and begin excavating. I buried my materials at a depth of three feet and it took one and a half 90-minute class periods for them to excavate all of the materials in the grave. You can dig shallower holes to speed up that process if you want. Once they have checked all of their evidence with you and you have confirmed that they have recovered everything, have them fill the hole back in so some poor groundskeeper doesn't fall in. The evidence should then be returned to the classroom for analysis. Teams should put all of their bones together to assemble one fairly intact skeleton and then properly identify the bones found and the characteristics of the person including age, race, height and gender. I have my students then, as teams, write a professional report detailing the excavation and their analysis of the bones. This can be done as homework.

Days 4 and 5

All of Day 4 and part of Day 5 will consist of notes on how drugs work in the body. I will cover absorption, distribution, metabolism and excretion. I will break up the notes with worksheets and practice problems on Volume of Distribution (see Appendix 5) and Calculating BAC (see Appendix 6). I model how to solve these types of problems for the students and them let them work with a partner to complete the worksheets. When I finish with this, I will have them complete the Virtual Autopsy activity (see Appendix 7) to give them some exposure to the work of the forensic pathologist.

Day 6

Today we will talk about how drug levels in the body change after death. We will also discuss postmortem drug testing methods. Students will be exposed to various types of drug spot tests and color tests by conducting a lab on the analysis of drugs. Fisher Scientific offers an excellent lab kit called "Kemtec Forensics Kit: Analysis of Drugs and Poisons" which includes several different tests for the students to perform on over-the-counter drugs as well as simulated marijuana and simulated LSD. The kits cost $281.00 from Fisher and work for class of approximately 24 students working in groups. The catalogue number for Fisher is S25702.

Days 7 and 8

Notes will be given on toxicity and LD50. Students will work in small groups to solve toxicity problems. (See Appendix 8) Students will prepare for and conduct an LD50 lab. (See Appendix 9) They will be given access to brine shrimp and several common

household chemicals. They will choose one chemical to test per lab group. They will develop their own procedures and explore the effects of various doses of the chemicals on their shrimp. They will turn in a formal lab report based on their experience.

Day 9

Students will be given a day to work on their book projects with their partners in class.

Day 10

Students will present their book projects in the form of a gallery walk. Half of the groups will be stationed at their project display while the other half rotates through the stations. Once everyone has seen all of the projects, students will switch places and the other half of the class will be able to rotate through the projects. Students should ask and get answers to questions at each station. They can then present their answers to the class as a summary of what they learned from the project. Time can also be taken during this class to review the unit material for the unit test.

Day 11

On the last day of the unit, administer an assessment in the format of your choice to assess mastery of unit material. I have attached one possible test as Appendix 10.

Appendix 1

Implementing Teaching Standards

The following standards for Honors Advanced Forensic Science are addressed in this unit:

Advanced Anthropology

HS-AFS-AA-1	Students will use environmental clues to locate unmarked graves.
HS-AFS-AA-2	Students will use correct techniques to excavate a grave
HS-AFS-AA-3	Students will properly recover a skeleton from an excavation site.

These standards ask students to learn about advances in forensic anthropology and to apply their knowledge to an actual unmarked burial site.

Advanced Pathology

HS-AFS-APa-2	Students will describe the steps in a death investigation
HS-AFS-APa-3	Students will explain how lab tests are used to determine factors that may have contributed to the death of a person

Students will learn how a death investigation is properly conducted and will explore various lab tests which screen for and confirm the presence of drugs in human tissues.

In addition, the following standards from Honors Forensic Science are addressed:

Pathology

HS-FS-Pa-1a	Students will describe the various procedures performed during an autopsy: a) record basic statistics of victim, b) record external observations, c) recover and store trace evidences, d) extract blood and urine sample, e) perform Y incision, f) remove breast plate, g) remove, examine and weigh major organs, h) examine skull and brain, and i) return bodily contents and stitch up skull and body chest.

Students will conduct a virtual autopsy to familiarize themselves with autopsy procedures and evidence collection.

Toxicology

HS-FS-T-2b	Students will be able to describe and perform the proper steps of collection and preservation of drug evidence in the field.
HS-FS-T-3a	Students will understand the process of isolating and identifying drugs, toxins and poisons in human tissue.

HS-FS-T-3b Students will understand and appreciate the difficulties in isolating drugs, toxins and poisons in human tissue.

Students will learn how drugs work in the human body by studying the principles of pharmacokinetics and pharmacodynamics. They will learn what samples should be taken for postmortem drug testing and will explore various lab tests to test for the presence of drugs and/or poisons. Students will also become familiar with the concept of toxicity and how to calculate lethal doses of drugs and poisons.

Appendix 2

LD50 Values for Common Substances

Substance	LS50 (mg/kg)	Notes
Botulin	0.00001	Formed by bacteria in improperly canned food
Cyanide	10	In apricot and cherry pits and used in industrial applications
Vitamin D	10	Essential to humans but toxic in amounts greater than found in normal diet
Nicotine	50	
Caffeine	200	
Acetylsalicylic acid	1,000	Aspirin
Sodium Chloride	3,000	Table salt
Ethanol	7,000	Drinking alcohol
Citric acid	12,000	Found in grapefruit, oranges and lemons
Sucrose	30,000	Common sugar
Acetaminophen	1944	Tylenol
Cannabidiol	980	
Ibuprofen	636	Advil or Motrin
Psilocybin	280	Found in mushrooms
Ketamine	229	Animal tranquilizer
Cocaine	96	
Methamphetamine	57	
Heroin	22	
LSD	16.5	
Strychnine	1-2	
Plutonium	0.32	
Fentanyl	0.30	
Polonium-210	0.0001	Radioactive metal
Ricin	0.022	

Appendix 3

The Poisoner's Handbook

Watch TedTalk by Deborah Blum called "Early forensics and crime-solving chemists."
https://www.youtube.com/watch?v=ORpeZP0jiCk

Discussion Questions:

- How does this book relate to the current state of the world, international relations, the press, or celebrities?
- What aspects of life in the early to mid-20th century made poisoning a likely way to commit murder?
- Why do you think many of the toxic products from that time are no longer available?
- What are some common products available now that are toxic? Do you think they will continue to be available in the future? Why or why not?
- Why do you think Marie Curie was so famous? How do you suppose her death affected the safety measures that future scientists would take?

Teacher's Guide to book
https://d43fweuh3sg51.cloudfront.net/media/media_files/amex26_doc_phguide.pdf
Contains instructions for lab simulations of the Prussian Blue Cyanide Test, the Pink Carbon Monoxide Test, the Photographic Radium Test Using UV-Sensitive Paper, Simulating the Arsenic "Silver Mirror"

The Poisoner's Handbook – Interactive: Tales from The Poisoner's Handbook
https://www.pbslearningmedia.org/resource/amex26-sci-novel/tales-from-the-poisoners-handbook/#.Wf9iQGiPLIU

TV Movie based on the book (1:48:32)
https://www.amazon.com/dp/B00HQXBWLU?ref_=imdbref_tt_wbr_piv&tag=imdbtag_tt_wbr_piv-20&ref_=nav_signin&

Appendix 4

List of Materials for Excavation Project

Shovels (square-tipped, if possible)
Trowels
Colanders (or for the ambitious, fine wire affixed to a frame for screening dirt)
Stakes
String
Index cards
Crime Scene Tape (optional)
Clip Boards
Plain paper
Graph paper
Measuring tapes
Brushes
Rakes
Rulers
Tape
Cameras
Evidence tags/Chain of Custody tags
Bones, at least 5 per grave (Ideally from the same skeleton so students can assemble the bones and identify characteristics of the person)
Other evidence to put in the holes: bullet casings, pennies, water bottles, cigarette butts, trash, etc…
Skeletal diagrams
Dental diagrams, if teeth are present
Copies of forms designed by students for their particular jobs

Appendix 5

Name: ___KEY_____Block: _____ Date: _____

Calculations with Volume of Distribution (V_d)

Every drug has its own volume of distribution which is fairly standard for that drug, although there are factors that can influence it. The basic equation for calculating concentration of a drug in blood plasma is:

$$C_p = \frac{X}{V_d}$$

Where C_p is the drug concentration in blood plasma, X is the amount of drug taken and V_d is the volume of distribution of the drug throughout the body.

Answer the following questions using your knowledge of drug distribution. Show your work!

1. The body of a 25-year-old woman who weighed 55 kg was found nude in a river, partially covered by a blanket. There were no signs of trauma and the autopsy showed that she did not drown. Toxicology findings were as follows: blood free morphine 0.45 mg/L and blood total morphine after enzyme hydrolysis 0.70 mg/L. Hair from the scalp was continuously positive along its entire length (20 cm) for morphine. The volume of distribution of morphine in the body is 3.3 L/kg. How much morphine did the woman take?

   Amount taken = V_d x weight x concentration
   = 3.3 L/kg x 55 kg x 0.45 mg/L
   = 81 mg

2. A car driven by a 31-year-old white male failed to follow the road curve, crossed over the centerline, and hit a car approaching from the opposite direction, resulting in two fatalities. This driver was a physician known to be despondent. The driver's blood specimen obtained at the hospital two hours after the crash contained 1.4 mg/L diazepam, 2.5 mg/L nordiazepam and 0.04 g/dL ethanol. The driver weighed 72 kg. The volume of distribution of diazepam is 1.1 L/kg. How much diazepam did the driver take?

   Amount taken = V_d x weight x concentration
   = 1.1 L/kg x 72 kg x 1.4 mg/L
   = 111 mg

3. What is the dose of gentamicin required to obtain a peak level of 20 mg/L in a 60 kg patient? The V_d for gentamicin is 0.25 L/kg.

   Amount taken = V_d x weight x concentration
   = 0.25 L/kg x 60 kg x 20 mg/L
   = 300 mg

4. An overdose patient arrives at the emergency room. Blood is drawn and it is determined that the patient has a blood plasma concentration of clonazepam (a sedative) of 4 mg/L. The V_d for clonazepam is 210 L. How much of the drug did the patient take?

   Amount taken = V_d x concentration
   = 210 L x 4 mg/L
   = 840 mg

5. What is the volume of distribution in L/kg for amphetamines if a 70 kg man takes 100 mg of the drug and within an hour has a blood plasma concentration of 0.29 mg/L?

   V_d = amount taken/plasma concentration
   = 100 mg / 0.29 mg/L
   = 345 L / 70 kg
   = 4.9 L/kg

6. What would you expect the blood plasma concentration of fentanyl to be if a 70 kg man took 2 g of fentanyl which has a volume of distribution of 3.6 L/kg?

   C_p = amount taken / V_d
   = 2 g / (3.6 L/kg x 70 kg)
   = 2 g / 252 L
   = 0.008 g/L

Name: _____Block: _____ Date: _____

Calculations with Volume of Distribution (V_d)

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Where C_p is the drug concentration in blood plasma, X is the amount of drug taken and V_d is the volume of distribution of the drug throughout the body.

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1. The body of a 25-year-old woman who weighed 55 kg was found nude in a river, partially covered by a blanket. There were no signs of trauma and the autopsy showed that she did not drown. Toxicology findings were as follows: blood free morphine 0.45 mg/L and blood total morphine after enzyme hydrolysis 0.70 mg/L. Hair from the scalp was continuously positive along its entire length (20 cm) for morphine. The volume of distribution of morphine in the body is 3.3 L/kg. How much morphine did the woman take?

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3. What is the dose of gentamicin required to obtain a peak level of 20 mg/L in a 60 kg patient? The V_d for gentamicin is 0.25 L/kg.

4. An overdose patient arrives at the emergency room. Blood is drawn and it is determined that the patient has a blood plasma concentration of clonazepam (a sedative) of 400 mg/L. The V_d for clonazepam is 210 L. How much of the drug did the patient take?

5. What is the volume of distribution for amphetamines if a 70 kg man takes 100 mg of the drug and within an hour has a blood plasma concentration of 0.29 mg/L?

6. What would you expect the blood plasma concentration of fentanyl to be if a 70 kg man took 2 g of fentanyl which has a volume of distribution of 3.6 L/kg?

Appendix 6

Name: _____KEY_____ Block: _____ Date: _____

Calculating Blood Alcohol Concentration Problems

The Widmark Formula for calculating the Blood Alcohol Concentration of a person based on the amount of alcohol they have consumed is:

BAC = {[alcohol consumed in g / (body weight in g x r)] x 100} - (elapsed time in hours x 0.015)

Where r is a gender constant (Male = 0.68, Female = 0.55)

A standard drink is 1.5 oz. of hard liquor, 12 oz. of beer or 5 oz. of wine. Based on the percent of alcohol in each type of drink, it can be determined that a standard drink contains 14g of alcohol.

For the following problems, use all available facts to determine the person's BAC.

1. A 120lb woman goes out with friends after work and drinks 3 glasses of wine over a 2-hour period. What is her BAC?

   Amount of alcohol consumed = # of standard drinks x 14g alcohol/drink
   = 3 x 14 = 42g alcohol

   Weight in g = 120lbs x 454g/lb = 54,480g

   BAC = {[42g/(54,480g x .55)] x 100} - (2hrs x 0.015)
   = [(42/29,964) x 100] - 0.03
   = (0.001401682 x 100) - 0.03
   = 0.14 - 0.03
   BAC = .11

2. A 210lb man consumes 9 beers in a 3-hour period. What is his BAC?

   Amount of alcohol consumed = # standard drinks x 14g alcohol/drink
   = 9 beers x 14g alcohol/beer
   = 126g alcohol consumed

   Weight in g = 210lbs x 454g/lb
   = 95,340g

   BAC = {[126g/(95,340 x 0.68)] x 100} - (3hrs x 0.015)
   = [(126g/64,831.2) x 100] - 0.045
   = (0.0019435087 x 100) - 0.045
   = 0.149

3. A 155lb woman drinks 4 glasses of wine and 3 shots of tequila over the course of 4 hours. What is her BAC?

Amount of alcohol = # drinks x 14 g alc/drink
= 7 x 14g
= 98g alcohol

Weight in g = 155lb x 454g/lb
= 70,370g

BAC = {[98g/(70,370 x 0.55)] x 100} - (4hrs - 0.015)
= [(98g/38703.5) x 100] - 0.06
= (0.0025320707 x 100) - 0.06
= 0.25 - 0.06
= 0.19

Name: _____ Block: _____ Date: _____

Calculating Blood Alcohol Concentration Problems

The Widmark Formula for calculating the Blood Alcohol Concentration of a person based on the amount of alcohol they have consumed is:

BAC = {[alcohol consumed in g / (body weight in g x r)] x 100} - (elapsed time in hours x 0.015)

Where r is a gender constant (Male = 0.68, Female = 0.55)

A standard drink is 1.5 oz. of hard liquor, 12 oz. of beer or 5 oz. of wine. Based on the percent of alcohol in each type of drink, it can be determined that a standard drink contains 14g of alcohol.

For the following problems, use all available facts to determine the person's BAC.

1. A 120lb woman goes out with friends after work and drinks 3 glasses of wine over a 2-hour period. What is her BAC?

2. A 210lb man consumes 9 beers in a 3-hour period. What is his BAC?

3. A 155lb woman drinks 4 glasses of wine and 3 shots of tequila over the course of 4 hours. What is her BAC?

Appendix 7

Name: _____Block: _____ Date: _____

Virtual Autopsy Worksheet

1. Go to www.australianmuseum.net.au/interactive-tools/autopsy/ . Answer the following questions.
    a. What is the 1st step in an autopsy?

    b. Where is the "Y" incision placed?

    c. What is the Rokitansky Method?

    d. How much do each of the organs weigh?

        1. lungs _____
        2. heart _____
        3. kidneys _____
        4. brain _____
        5. liver _____

2. Go to www.le.ac.uk/pa/teach/va/titlpag1.html. Pick a case to work. Read the Case History. Click on the various body systems on the interactive cadaver to gather information about the deceased. When you have gathered enough information, click on "Cause of Death" to see if you are ready to become a forensic pathologist! Describe the case history, body systems and cause of death for the case you chose.

Appendix 8

Name: _____KEY_____ Block: _____ Date: _____

Toxicity Calculations

1. Based on LD50 data, how many cups of coffee would it take to kill an average human of your size? Assume that a cup of coffee contains 90 mg of caffeine.
   Answers will vary with size.
   Lethal Dose of Caffeine in mg = LD50 in mg/kg x weight in kg
   Lethal Dose of Caffeine in mg / 90 mg per cup = _____ cups of coffee

2. Using your answer from #1, if you were to drink one cup of coffee per day for that number of days, would you likely overdose on caffeine? Why or why not?
   No because the body can eliminate the caffeine in one cup of coffee per day before the caffeine reaches toxic levels in the body.

3. If you could drink the number of cups of coffee from #1 all at once, would you be guaranteed to die? Why or why not?
   No. Everyone is different and different factors will affect each person's ability to eliminate the caffeine.

4. What is the most important assumption we make when we use LD50 data to estimate a lethal dose in humans?
   That humans will respond in the same way as rats do to the compounds

5. Based on LD50 data, calculate the amount of cyanide it would take to kill a 180-pound man.
   180 lb man = 81.6 kg x 10 mg/kg (from LD50 Table) = 816 mg

6. Based on LD50 data, calculate how many standard-sized alcoholic drinks it would take to kill a 130-pound woman. Assume a standard drink contains 14 g of alcohol.
   130 lb = 58.9 kg x 7,000 mg/kg = 412,3000 mg
   412,300 mg x 1 g/1000 mg = 412.3 g alcohol
   Standard drink contains 14 g alcohol so 412.3 g / 14 g = 29.5 drinks

Name: _____ Block: _____ Date: _____

Toxicity Calculations

1.Based on LD50 data, how many cups of coffee would it take to kill an average human of your size? Assume that a cup of coffee contains 90 mg of caffeine.

2.Using your answer from #1, if you were to drink one cup of coffee per day for that number of days, would you likely overdose on caffeine? Why or why not?

3.If you could drink the number of cups of coffee from #1 all at once, would you be guaranteed to die? Why or why not?

4.What is the most important assumption we make when we use LD50 data to estimate a lethal dose in humans?

5.Based on LD50 data, calculate the amount of cyanide it would take to kill a 180-pound man.

6.Based on LD50 data, calculate how many standard-sized alcoholic drinks it would take to kill a 130-pound woman. Assume a standard drink contains 14 g of alcohol.

Appendix 9

Names: _____ Block: ____ Date: _____

Brine Shrimp LD50 Toxicity Lab

Brine Shrimp are marine crustaceans, also commonly known as "sea monkeys". Brine shrimp eggs can remain in total stasis for two years while in dry oxygen-free conditions (called cryptobiosis). Eggs hatch after being placed in salt water for a few hours.

The Lethal Dose 50 (LD50) is a test that is used to find out what concentration of a particular substance will kill 50% of a population.

In this lab, you will pick a substance that you think will kill the brine shrimp. You will test this substance to find the LD50.

Hypothesis:

Materials:
Toxic agent
Salt Water
Live brine shrimp
10 mL graduated cylinder
Pipettes
5 Test tubes

Procedure:

1. Fill in the chart below:

Independent Variable (IV)	Constants (C)	Dependent Variable (DV)

2. Setting up serial dilution:

    a. Label 5 test tubes: #1, #2, #3, #4, #5
    b. Add 9 mL water and 1 mL of your substance into test tube #1.
    c. Add 9 mL water and 1 mL from test tube #1 into test tube #2.
    d. Add 9mL water and 1 mL from test tube #2 into test tube #3.
    e. Add 9mL water and 1 mL from test tube #3 into test tube #4
    f. Add 10 mL water into test tube #5. This is your control

3. Add 10 brine shrimp to each test tube
4. Allow experiment to sit overnight
5. Count remaining brine shrimp and record your results.

Data:

Solution	Concentration	Number of dead brine shrimp
#1		
#2		
#3		
#4		
#5	Control	

Graph:

Conclusions:

1. What is the LD50 of your substance on brine shrimp?

2. Identify two experimental errors.

3. Do you think your results are valid? Why or why not?

4. Ask other students in the class and find out which substance had the worst LD 50 levels. What does this tell you about that substance? Will that information change what you eat or how you live?

5.Are there any ethical issues in using animals for testing LD 50 levels? If so, where do you draw the line?

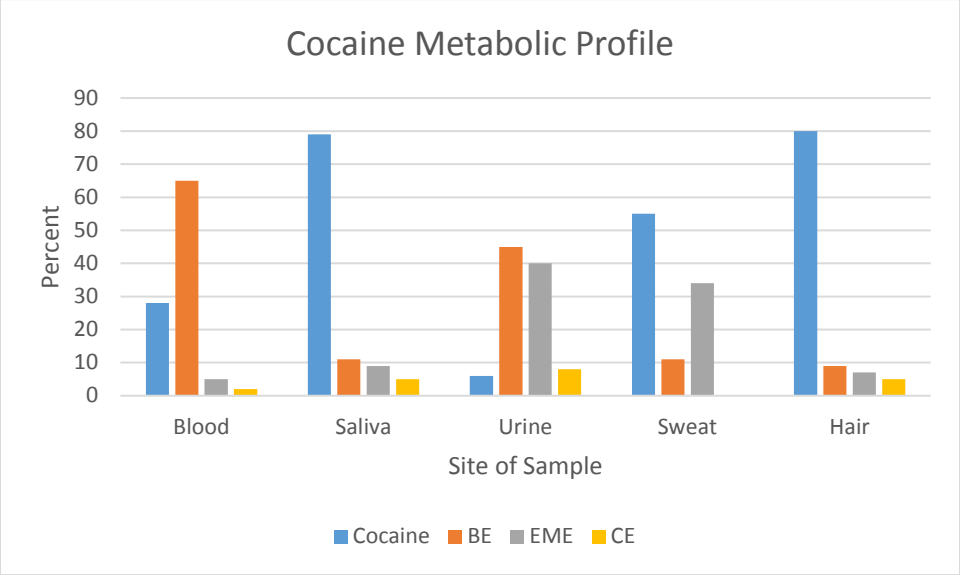
6.What information is gained through LD50 testing? In your opinion, is the information we get from these tests worth the deaths of the organisms tested?

Appendix 10

Name: _____KEY_____ Block: _____ Date: _____

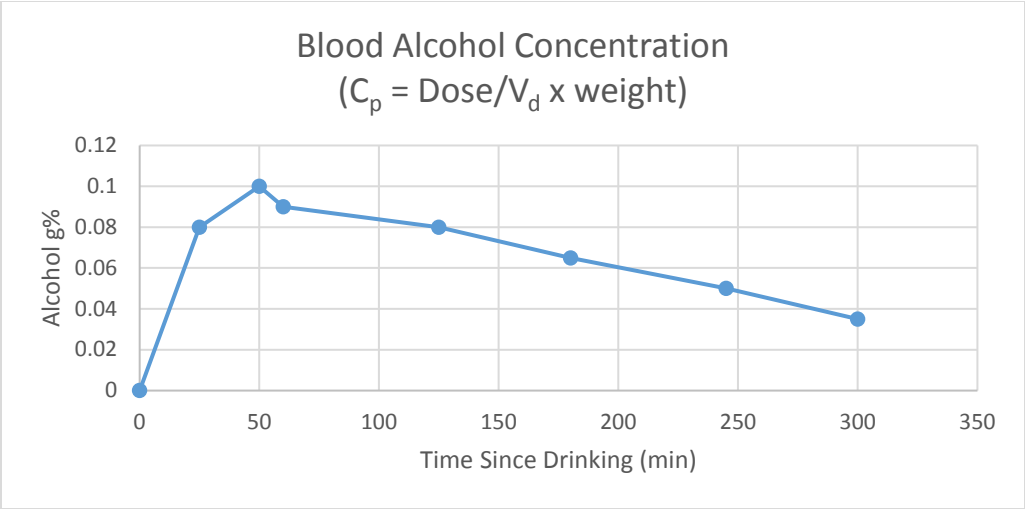
Unit Assessment

Below is a graph showing the metabolic profile of cocaine when tested in various body tissues. BE, EME and CE are metabolites of cocaine. Use the graph to answer the questions below.



1. Which body tissue will show the largest percentage of free cocaine? Hair
2. Which body tissue shows the most even production of cocaine metabolites? Urine
3. From which body tissue does cocaine disappear the fastest? Urine
4. Which body tissue will show the presence of cocaine the longest? Hair

Below is a graph showing the concentration of alcohol in the body over time. Use the graph below to answer the following questions.



5. What is the peak blood alcohol concentration? 0.1
6. At what time after drinking does the BAC peak according to the graph? 50 min
7. At what time after drinking did the body's BAC return to under the legal limit for driving? After approximately 125 min
8. Assuming the curve of the graph continues at a constant rate, estimate the time at which the body would be completely clear of alcohol. Approximately 425 min

9. A 33-year-old white female was admitted to the hospital after taking 60 digoxin tablets. Her blood concentration at admission was 45 ng/mL digoxin. Six hours later, her digoxin concentration was 18 ng/mL. At autopsy 27 hours after her death, the blood digoxin concentration was 36 ng/mL. Explain how this is possible.
   The increase in digoxin concentration is due to the postmortem redistribution of the drug from the heart muscle where it acts into the blood vessels near the heart.

10. The body of a 20-year-old male weighing 70 kg was found in an apartment. There was a spoon and a lighter on the table. At autopsy, the medical examiner found numerous needle marks on the man's arms. The toxicology results indicated a concentration of free morphine of 0.54 mg/L. The volume of distribution of morphine is 3.3 L/kg.

    a. Describe all of the indicators that this might be a drug overdose found at the scene and at the autopsy.
       The spoon and lighter are paraphernalia used when cooking drugs for injection. The needle marks on his arms indicate that he may have been an intravenous drug user.

b. Calculate the amount of morphine the man injected before his death.
Amount = V_d x weight x concentration
= 3.3 L/kg x 70 kg x 0.54 mg/L
= 124.7 mg

11. A 60 kg woman consumed 3 glasses of wine and 3 shots of tequila over the course of 3 hours. Calculate her BAC. The gender constant for females is 0.55.
BAC = {[amount of alcohol / (weight x r)] x 100} – (time elapsed x 0.015)
= {[(6 drinks x 14 g alcohol/drink) / (60 kg x 1000g/kg x 0.55)] x 100} – (3 x 0.015)
= [(84 / 33,000) x 100] – (0.045)
= (0.00254 x 100) – 0.045
= 0.254 – 0.045
= 0.209

12. Calculate the amount of cocaine it would take to kill a 70 kg male. The LD50 of cocaine is 96 mg/kg.
70 kg x 96 mg/kg = 6,720 mg or 6.72 g

13. What is the most important assumption we make when we use LD50 data to estimate lethal doses in humans?
That humans will respond in the same way to substances at rats

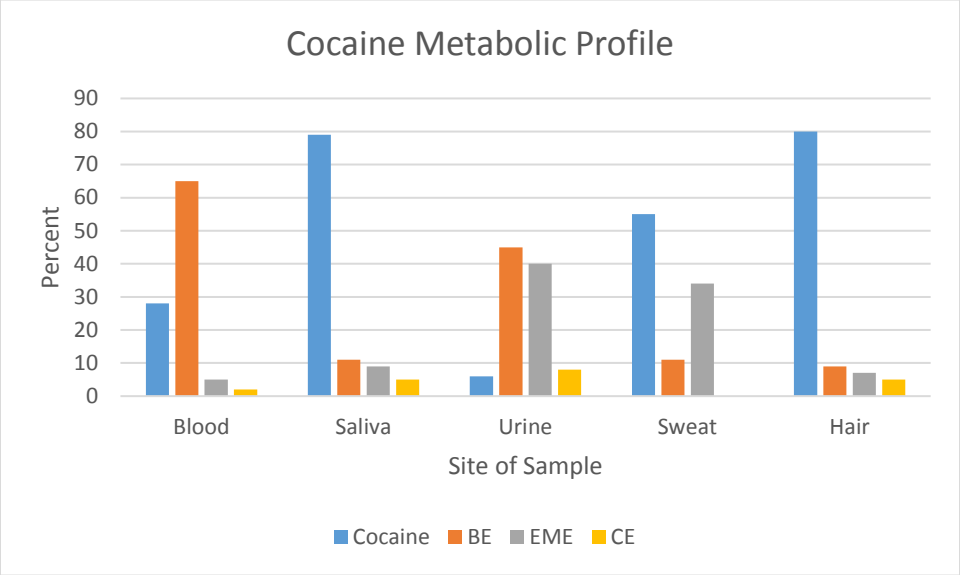
14. Calculate the number of drinks it would take to kill a 50 kg woman. The LD50 of alcohol is 7,000 mg/kg. A standard drink contains 14 g of alcohol.
Lethal dose = 7,000 mg/kg x 50 kg = 350,000 mg
350,000 mg x 1 g/1000 mg = 350 g
350 g / 14 g/drink = 25 drinks

15. Briefly describe the four processes of pharmacokinetics.
Absorption is the process whereby drugs enter the bloodstream. Distribution is the process whereby drugs move throughout the body. Metabolism is the process whereby drugs are transformed to be more water soluble to aid in elimination from the body. Excretion is the final removal of the drug from the body.

Name: _____ Block: _____ Date: _____

Unit Assessment

Below is a graph showing the metabolic profile of cocaine when tested in various body tissues. BE, EME and CE are metabolites of cocaine. Use the graph to answer the questions below.



1.Which body tissue will show the largest percentage of free cocaine?
2.Which body tissue shows the most even production of cocaine metabolites?
3.From which body tissue does cocaine disappear the fastest?
4.Which body tissue will show the presence of cocaine the longest?

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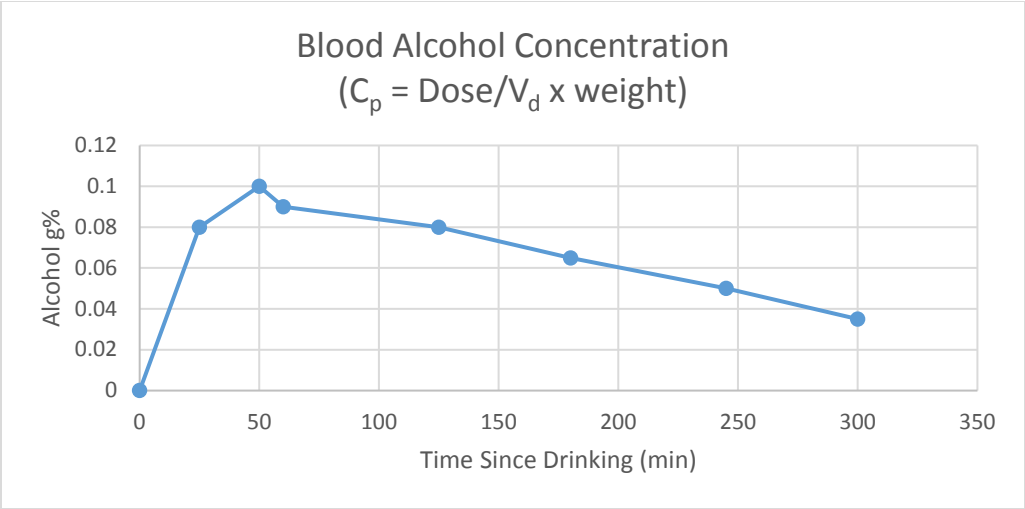

5.What is the peak blood alcohol concentration?
6.At what time after drinking does the BAC peak according to the graph?
7.At what time after drinking did the body's BAC return to under the legal limit for driving?
8.Assuming the curve of the graph continues at a constant rate, estimate the time at which the body would be completely clear of alcohol.

9.A 33-year-old white female was admitted to the hospital after taking 60 digoxin tablets. Her blood concentration at admission was 45 ng/mL digoxin. Six hours later, her digoxin concentration was 18 ng/mL. At autopsy 27 hours after her death, the blood digoxin concentration was 36 ng/mL. Explain how this is possible.

10.The body of a 20-year-old male weighing 70 kg was found in an apartment. There was a spoon and a lighter on the table. At autopsy, the medical examiner found numerous needle marks on the man's arms. The toxicology results indicated a concentration of free morphine of 0.54 mg/L. The volume of distribution of morphine is 3.3 L/kg.

a. Describe all of the indicators that this might be a drug overdose found at the scene and at the autopsy.
b. Calculate the amount of morphine the man injected before his death.

11.A 60 kg woman consumed 3 glasses of wine and 3 shots of tequila over the course of 3 hours. Calculate her BAC.

12.Calculate the amount of cocaine it would take to kill a 70 kg male. The LD50 of cocaine is 96 mg/kg.

13.What is the most important assumption we make when we use LD50 data to estimate lethal doses in humans?

14.Calculate the number of drinks it would take to kill a 50 kg woman. The LD50 of alcohol is 7,000 mg/kg. A standard drink contains 14 g of alcohol.

15.Briefly describe the four processes of pharmacokinetics.

Student Resources

Blum, Deborah. *The Poisoner's Handbook: Murder and the Birth of Forensic Medicine in Jazz Age New York*. Penguin Press, 2011. Fantastic book for an in-class project. Covers the history of the development of forensic toxicology with many case studies and fascinating stories.

Burns, Karen Ramey and Joanna Wallington. *Forensic Anthropology Training Manual*. 3rd ed. Boston: Pearson, 2013. Excellent handbook with background information and "how-to" explanations. Covers the skeleton, odontology, determining characteristics, excavations and report writing.

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Teacher Resources

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four processes involved in the movement of drugs throughout the body. Lasts 16:13 minutes.

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Notes

[1] Kennedy, *Interpreting postmortem drug* analysis, 56.
[2] Ferner, *Post-mortem clinical pharmacology*, 433.
[3] *CSI:GeoPhysics.*
[4] Everts, *Scientists search for death's aroma*, 16-18.
[5] Page, *LABRADOR: New alpha dog in human remains detection?*
[6] Hand-Held Analyzer Quickly Detects Buried Human Remains.
[7] Burns and Wallington, *Forensic Anthropology Training Manual*, 244.
[8] Ibid, 253.
[9] Levine, Principles of Forensic Toxicology, 47.
[10] Ibid.
[11] A xenobiotic is a substance that is foreign to the body.
[12] Levine, Principles of Forensic Toxicology, 47.
[13] Ibid.
[14] Ibid.
[15] Material taken from Levine, Principles of Forensic Toxicology, 47-8.
[16] Levine, Principles of Forensic Toxicology, 48.
[17] Levine, Principles of Forensic Toxicology, 47.
[18] Levine, Principles of Forensic Toxicology, 49.
[19] Ibid.
[20] Ibid.
[21] https://en.wikipedia.org/wiki/Volume_of_distribution
[22] https://www.wikihow.com/Calculate-Blood-Alcohol-Content-(Widmark-Formula).
[23] *Alcohol (BAC, Gender, Etc…).*
[24] Levine, Principles of Forensic Toxicology, 50.
[25] *Pharmacokinetic Processes: Metabolism.*
[26] Ibid.
[27] Levine, Principles of Forensic Toxicology, 52.
[28] Ibid.
[29] Ibid.
[30] "Pharmacokinetics for Students: Absorption, Distribution, Metabolism and Excretion – Lecture 1."
[31] Ferner, 431.
[32] Ibid, 433.
[33] Ibid.
[34] Cook, "Estimating antemortem drug concentrations."
[35] Kennedy, Interpreting postmortem drug analysis.
[36] Ferner, 435.
[37] Ibid, 431.
[38] Ibid, 432.
[39] Ibid, 433.
[40] https://en.m.wikipedia.org/wiki/Toxicity_category_rating.

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