

## Forensic Toxicity Testing

### I. Forensic toxicity testing

#### A. Analytic chemistry melded with organ damage consistent with the compound and concentration found (consonance of data)

1. Most intentional poisoning deaths = suicide; most unintentional poisoning deaths from overdose of opioids and cocaine; toxicity from ethanol, drugs of abuse, overuse of medications lead car crash causes; unintentional nonfatal poisonings caused by opioids > benzodiazepines
2. Most exposures were oral
3. Sampling of tissues

- a. Living being is preferred as death results in tissue destruction, possible loss of toxicant, and redistribution of toxicant (from organ to plasma resulting in false poisoning indication if plasma is the key item utilized)

For humans: take 15 mL of blood for ethanol/toxicants

Take 30 mL urine for metabolites

Hair samples (pulled not cut) for drugs of abuse (e.g., methamphetamine in child's hair from house manufacturing this drug) → extraction with HCl → enzymatic treatment (Pronase or β-glucuronidase) → hot CH<sub>3</sub>OH extraction → hot NaOH disintegration and extraction

Metals → urine, blood, and hair samples + possibly fingernails (As especially)

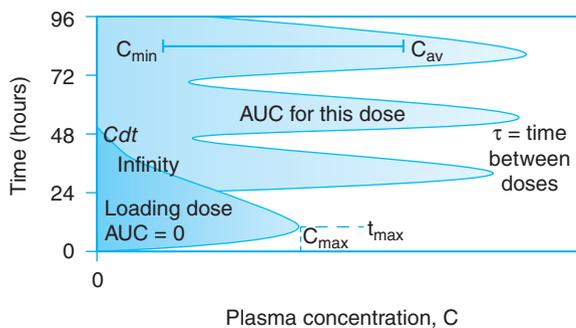
Teeth for chronic exposure to Pb in children

#### (1) Therapeutic drug monitoring

- (a) Required for highly lipophilic medications or pharmacokinetics, vary widely by patient
- (b) Also may be a function of a larger loading dose, long half-life, and narrow therapeutic range for action vs. toxicity (digitalis as loading dose needed to establish 0.5–1.5 or 2.0 ng/mL therapeutic range, 2 ng/mL toxicity, 1 h to achieve active plasma levels when taken orally, and a  $t_{1/2} = 160$  h + interacts with many medications including Ca<sup>2+</sup>-channel blockers, class III antiarrhythmic amiodarone, β-blockers, and NSAIDs)—see Table 14-2
- (c)  $C_{\text{trough}}$  (also known as  $C_{\text{min}}$ ) works for HIV medication atazanavir or cancer chemotherapy medication methotrexate—see figure

### CONCEPTUALIZING TOXICOLOGY 14-1

(d) For compounds such as the immunosuppressant mycophenolate mofetil used for kidney transplantation, the trough value doesn't correlate with dose or therapeutic effect so concentration at 2 h (C<sub>2</sub>) following dosing sampled



- b. For dead people or animals, antemortem or postmortem collection of as many fluids as possible to reach results (peripheral blood, aqueous humors, urine, stomach contents, etc.), analyzing for original compound + metabolites:  
50 g of brain, liver, kidney; 25 mL of heart blood; 10 mL of peripheral blood; and all available vitreous humors, bile, urine, and gastric contents  
For specific toxicants, lung, intestine, or other specimens may be taken from other organs or larvae feeding on corpses as was done for malathion.
4. Analysis of tissues (classic analytic chemistry)
  - a. Acid digestion → steam → volatiles (acetaldehyde, acetone, CO, CN, CH<sub>3</sub>OH, CH<sub>3</sub>CH<sub>2</sub>OH, isopropanol, phenol, or anions such as Br<sup>-</sup>, F<sup>-</sup>, oxalate)  
Acid fraction → leaches metals → atomic absorption (AA) or inductively coupled plasma emission spectroscopy (ICP)
  - b. Base digestion → aqueous phase-acidified + organic solvent → cleanup of proteins → barbiturate analysis  
Base digestion → organic phase-acidified → aqueous (made basic and contains basic medications such as amphetamine) + organic phases (contains neutral medications such as simvastatin)
5. Analysis of tissues (modern extraction linked to GC-MS, LC-MS, or LC-[tandem] MS/[MS])
  - a. Tissue represents the aqueous phase continuously extracted in the presence of inert diatomaceous earth by liquid-liquid chromatography extraction wells robotically; organic eluate is evaporated and reconstituted for electrospray (tandem) LC-MS/MS analysis.
6. Rapid screening tests—see Table 14-1
  - a. Drugs of abuse are detectable in urine up to a few hours to days following use, while peptide hormones are undetectable in urine; injected steroids may be detectable for a month or more following use.
  - b. Certain standards of indications for human urine involve correct urine temperature, pH, creatinine, specific gravity, and human IgG.
  - c. Adulterants involve changing pH, oxidizing agents, fixatives (to ruin immunoassays), while diuretics are used to remove metabolites more rapidly prior to testing.
  - d. Hair samples have ability to confirm use of drugs of abuse up to 90 days following use.
  - e. Usual postmortem examination for toxicants—Figure 14-1; first considerations are CO poisoning (COHb), CN (from fire inhalation or plant ingestion), drugs of abuse or prescription medications, hypoglycemia (insulin overdose or other causes), uncontrolled diabetes mellitus, ethanol

### CONCEPTUALIZING TOXICOLOGY 14-1 (continued)

Blood → COHb, CN, HbA<sub>1c</sub>, drug analyses, ethanol  
 Urine → drug (metabolites), ethanol, glucose  
 Vitreous humor → ethanol, glucose  
 Bile → RIA for drugs where urine can't be collected  
 Gastric contents → drugs  
 Liver, skeletal muscle, spleen, lung, kidney, brain, heart → drugs, ethanol  
 CSF (if enough available) → drugs, ethanol  
 Problem is organ most protected on plane impact is kidney (80% available), while urine is not (80% unavailable)—see Figure 14-2.

#### B. Signs/symptoms of exposure

1. Toxidromes—symptoms consistent with exposure to a given agent
  - a. Ethanol use—ataxia, slurred speech, nystagmus on light exposure, etc.
  - b. Hg—triad of tremors, erethism, and gingivitis
  - c. Pediatric toxidromes and agents
    - (1) Respiratory—difficult breathing for botulinum toxin, d-tubocurarine, organophosphates, carbamates, strychnine, CO, CN, metabolic acidosis, liver failure, heart failure, methemoglobinemia (metHb), salicylates
    - (2) Hypoxia—CO, NO<sub>3</sub>/NO<sub>2</sub>-induced metHb formation (of G-6-PDH deficiency), CN
    - (3) Wheezing—β-adrenergic antagonists, Cl (irritant) gas, hydrocarbons, isocyanates, organophosphates, carbamates, smoke inhalation, sulfites in food
    - (4) Osmolarity gap—acetone, ethanol, ethyl ether, ethylene glycol, isopropanol, mannitol, methanol, renal failure, ketoacidosis
    - (5) Anion gap—acetaminophen, β-adrenergic medications, CO, CN, Fe, isoniazid, salicylates, theophylline, toxic alcohols, valproic acid
    - (6) Neuromuscular—antipsychotics, metoclopramide, amphetamines, anticholinergics, antihistamines, cocaine, γ-hydroxybutyrate, SSRIs and tricyclic antidepressants, malignant hyperthermia, phencyclidine (PCP)
    - (7) CNS depression—opiates, ethanol/organic solvents, sedative-hypnotics, tricyclic antidepressants
    - (8) Coma—alcohols/organic solvents, anticholinergics, As, β-adrenergic antagonists, cholinergic agents, CO, Pb, Li, opioids, PCP, phenothiazine antipsychotics, salicylates, sedative-hypnotics, tricyclic antidepressants
    - (9) Miosis (pupil constriction)—opoids, cholinergic agents, clonidine (α<sub>2</sub> adrenergic agonist), nicotine, phenothiazines, PCP
    - (10) Mydriasis (pupil dilation)—anticholinergics, glutethimide, meperidine, sympathomimetics, withdrawal from alcohol or opoids
    - (11) Seizures—amphetamines, cocaine, caffeine, theophylline, tricyclic antidepressants, venlafaxine, phenothiazines and butyrophenones, camphor, organophosphates, carbamates, ethylene glycol, isoniazid, meperidine, methanol, salicylates, withdrawal from depressants
    - (12) Serotonin syndrome—monoamine oxidase inhibitors (MAOIs) + SSRIs
    - (13) Sleepiness—antihistamines, any sedative hypnotic, alcohols, γ-hydroxybutyrate, tricyclic antidepressants, opoids, CO, CN, hypoglycemic agents
    - (14) Withdrawal from depressants opposite to withdrawal from stimulants (effects of withdrawal opposite to action of agent)
    - (15) Hyperthermia—serotonin syndrome, anticholinergics, MAOIs, metals, PCP, phenothiazines, salicylates, sympathomimetics, withdrawal from CNS depressants, malignant hyperthermia (halothane or succinyl choline)

#### CONCEPTUALIZING TOXICOLOGY 14-1 (continued)

- (16) Hypothermia— $\beta$ -blockers, CO, cholinergic agonists, ethanol, hypoglycemia-inducing agents, sedative-hypnotics
  - (17) Cardiovascular
    - (a) Hypertension—glycyrrhizin, mineralocorticoids,  $\alpha$ -1 agonist, Cd
    - (b) Hypotension—CO, CN, hemorrhagic agents, Fe, opioids, nitrites, phenothiazine, sedative-hypnotics, tricyclic antidepressants, theophylline
    - (c) Bradycardia— $\beta$ -blockers,  $\text{Ca}^{2+}$ -channel blockers, cardiac glycosides, clonidine, CN (high concentration), Li, nicotine, opioids, carbamates, organophosphates, parasympathomimetics
    - (d) Tachycardia—amphetamines, antihistamines, caffeine, clonidine, cocaine, theophylline, CO (lower concentrations), CN (lower concentrations),  $\text{H}_2\text{S}$ , anticholinergics, ethanol, PCP, sympathomimetics, withdrawal from CNS depressants, theophylline, thyroid hormone
    - (e) Torsade de pointes (dangerous ventricular arrhythmia)—amiodarone, As, chloroquine, quinine, quinidine, organophosphates, tricyclic antidepressants
  - (18) Hepatic—acetaldehyde, acetaminophen, aflatoxin, *Amanita phalloides*,  $\text{CCl}_4$ , other chlorinated hydrocarbons, halothane, phenol, phosphorus, valproic acid
  - (19) Renal—Hg, Au, Cd, statins via muscle wasting and creatinine formation
  - (20) Skin—Sulfonamides, aromatic antiepileptics, lamotrigine, penicillins, doxycycline, nevirapine, strong acids and bases, anilines, CO, cyanide, strychnine, metals
2. Toxicoproteomics
- a. Develop biomarkers for organ = specific toxicity
  - b. Organ (e.g., liver) and plasma must be analyzed to detect proteins and peptides released or leaked from the organ
  - c. Use five levels of analysis:
    - (1) Match DNA microarrays with altered protein profiles
    - (2) Compare affected pathways from gene transcripts and proteins responsible for activation or repression events
    - (3) Posttranslational altered proteins analysis as reflective of gene expression of kinases, proteases, phosphorylases, conjugation enzymes
    - (4) Protein trafficking and signaling pathway determination in nuclei, mitochondria, endoplasmic reticulum, plasma membrane and cytosol
    - (5) Kinetic protein analysis of serum proteome over pretoxic, toxic, and recovery periods

### CONCEPTUALIZING TOXICOLOGY 14-1 (continued)

This section includes forensic charts for each chapter describing an organ system. The most likely toxicants that primarily damage each organ are included in each chart. As tempting as it is to give a “laundry list” of toxicants that damage an organ, there usually is an organ through which the lethal effects are manifested. For example, an extremely high concentration of heavy metals given orally may strip off the cells of the intestinal tract and lead to hemorrhaging and death.

A lower oral dose may damage the liver, which stores metals. A lower dosage yet would likely damage the kidney, as this is the excretory organ. A lower dose might also cause central nervous system (CNS) damage. This forensic approach indicates that toxicant damage to organs may not be a lethal or even extremely toxic event with irreversible damage. All toxicants that affect these systems are characterized based on the ability to do significant damage or have the

toxicant altered in a way that damages another organ (e.g., liver detoxication leading to kidney damage). This might appear to be a chapter on analytical chemistry, and in some ways it is an extension of this area. In another sense, there must be consonance of data. That means that the amount or concentration of toxicant at the target organ, the damage found, and the symptoms prior to death should all confirm the toxicity. For example, if a person had a high amount of cyanide in the intestinal tract but showed no cyanosis, this would not likely be the cause of death. A blunt trauma to the head may be more indicative of the brain damage that was found. A person found under water with no water in the lungs did not likely drown but died prior to that point. Similarly, it is necessary to have data that consistently point to the cause of death.

## Most Likely Suspects

The toxicants that are usually tested in clinical or forensic toxicology laboratories are those most likely to kill or significantly affect function in humans. These toxicants have government programs to limit exposure such as drugs of abuse, overdose of medications, ethanol, carbon monoxide, lead poisoning, and a series of other toxicants. Most of the agents can be found in common use in the households of many Americans. The Centers for Disease Control (CDC), in 2008 reported that > 41,000 people died from poisoning. For the first time since 1980, poisoning was the leading cause of injury death and tripled over a 30 year period. Medication and illegal drug deaths in that period increased from a bare majority (60%) to almost the sole cause of poisoning deaths (90%; Warner, 2011). These mainly drug-related poisoning deaths in the U.S. are still mainly unintentional, while the remainder result from suicides or undetermined intent (CDC, 2015). Motor vehicle crashes were the first in unintentional deaths, and those related to intoxication from alcohol, drugs of abuse, overuse of medications, and so forth appear to lead that list. It is of interest that unintentional poisonings of people between the ages of 35 and 54 exceeded deaths from car accidents. The overwhelming majority of the 18% of intentional deaths from poisoning

in 2005 (5,833 people) were suicides rather than homicides (89 homicides). Ninety-five percent of all unintentional and undetermined deaths were related to opioid pain medication overdose followed by the drugs of abuse cocaine and heroin. Unintentional nonfatal poisonings came mainly from opioids followed by benzodiazepines (GABAergic sleeping pills). A more defined accounting for the year 2007 was a publication put forward from the American Association of Poison Control Centers (Bronstein et al., 2008). In 2007, 2,482,041 human exposures were reported (8.1/1,000 population). Nearly 65% of those calls were for children. Greater than 78% were oral exposures. Most cases resulted in no effect or minimal toxicity. Lethality was mostly experienced in adults over 19 years of age (0.2% of the 860,692 cases for that age group).

In 2007, 131,744 nonhuman exposures were reported, primarily for dogs (118,371) followed by cats (11,818). The top 25 exposures reported to U.S. poison control centers were analgesics (12.5%); cosmetics and personal care products (9.1%); household cleaning supplies (8.7%); sedative-hypnotics and antipsychotic medications (6.2%); foreign bodies, toys, and miscellaneous items (5.1%); topical preparations (4.5%); cold and cough medicines (4.5%); antidepressants (4.0%); pesticides (3.9%); cardiovascular medications (3.5%); alcohols (3.3%); antihistamines (3.2%); food products and food poisonings (3.1%); bites and envenomations (3.1%); antimicrobials (2.7%); vitamins (2.7%); plants (2.4%); hormones and hormone antagonists (2.2%); gastrointestinal preparations (2.2%); hydrocarbons (2.0%); chemicals (2.0%); stimulants and street drugs (1.9%); anticonvulsants (1.7%); arts, crafts, and office supplies (1.6%); and fumes, gases, and vapors (1.6%). People over 19 years of age were most likely exposed to cosmetics and personal care products (20.0%); cleaning supplies (14.3%); analgesics (13.4%); foreign bodies, toys, and miscellaneous items (11.1%); topical preparations (10.1%); cold and cough medicines (7.6%); vitamins (5.7%); pesticides (5.2%); plants (4.9%); antihistamines (4.6%); gastrointestinal preparations (4.3%); antimicrobials (4.0%); arts, crafts, and office supplies (3.4%); hormones and hormone antagonists (3.0%); cardiovascular medications

(2.8%); electrolytes and minerals (2.8%); alcohols (2.7%); food products and food poisonings (2.3%); deodorizers (2.0%); dietary supplements, herbals, and homeopathic preparations (2.0%); asthma therapies (1.9%); hydrocarbons (1.8%); other or unknown nondrug substances (1.8%); sedative-hypnotics and antipsychotic medications (1.7%); and antidepressants (1.6%). Exposures for children under 6 years of age involve cosmetics and personal care products (10.7%); cleaning supplies (7.6%); analgesics (7.2%); foreign bodies, toys, and miscellaneous items (6.0%); topical preparations (5.4%); cold and cough medicines (5.4%); vitamins (3.1%); pesticides (2.8%); plants (2.6%); antihistamines (2.5%); gastrointestinal preparations (2.3%); arts, crafts, and office supplies (2.2%); hormones and hormone antagonists (1.8%); cardiovascular medications (1.5%); electrolytes and minerals (1.5%); alcohols (1.5%); food products and food poisoning (1.3%); deodorizers (1.2%); dietary supplements, herbals, and homeopathic preparations (1.1%); asthma therapies (1.0%), hydrocarbons (1.0%); other or unknown nondrug substances (1.0%); sedative-hypnotics and antipsychotic medications (0.9%); and antidepressants (0.9%).

Which ones are the most lethal? For 2007, the most fatalities were reported for sedative-hypnotics and antipsychotic medications (377 people); opioids (331); antidepressants (220); acetaminophen in combination (208); cardiovascular medications (203); stimulants and other street drugs (188); alcohols (170); acetaminophen only (140); anticonvulsants (99); fumes, gases, and vapors (80); cyclic antidepressants (80); muscle relaxants (70); antihistamines (69); aspirin alone (63); chemicals (45); unknown drug (44); other nonsteroidal anti-inflammatory drugs (NSAIDs; 44 people died); oral hypoglycemic (36); automotive, aircraft, and boat products (28); miscellaneous drugs (21); antihistamine/decongestants without phenylpropanolamine (21); hormones and hormone antagonists (20); anticoagulants (20); and diuretics (16). (Bronstein et al., 2012)

A real forensic toxicologist is not a television crime scene analyst looking at rare chemicals used by a clever murderer. They usually are employed to do head space analysis of blood

collected by a police officer for a person arrested for driving under the influence of ethanol on a weekend or holiday. However, increasing numbers of children are having hair samples pulled from their scalps using gloves to detect methamphetamine as evidence against their parents (for having a drug lab in home). Note that it is going to be important to match signs and symptoms of exposure to sampling techniques and handling/storage of samples, and finally methods of analysis. Forensic analysis means a legal analysis that will be scientifically sound, meet specifications for chain of evidence and chain of custody (knowing each person responsible for the sampling or sample throughout the analysis), be free from contamination by samplers and analysis group, have confirming tests, and be consistent with medical knowledge as to cause of poisoning or death. The sections that follow examine each area to show the extent of scientific detective work that should ultimately yield a report on the agent or mixture of agents consonant with the observed toxicity.

## Signs/Symptoms of Exposure

Some toxicities are easily diagnosed, because they come with a variety of unmistakable symptoms. Others are more difficult to assess, because the symptoms are common for a variety of agents. An example of a toxicology assessment is when a police officer stops a car for erratic driving, and then shines a light into the driver's eyes. If the eyes vibrate (nystagmus) in the light, the officer will assume ethanol intoxication and test for ataxia via a walking test and having the driver touching his finger to his nose. If the eyes appear too constricted, the officer will assume that opiates may be involved. Toxidromes are a set of symptoms associated with a given agent or agents that may be used to evaluate a poisoned animal or person. Some of the most common toxidromes are for stimulants, sedative-hypnotic medications, opiates, anticholinergics, and cholinergic agents (Tomassoni et al., 2015). Certain toxidromes have been noted historically and in novels. For example, the mad hatter in Lewis Carroll's *Alice's Adventures in Wonderland* has the syndrome associated with mercury poisoning

due to spraying of felt hats with mercury nitrate to preserve the fiber. The set of symptoms fit the fictional mad hatter with excitability (erethism), gum disease (gingivitis), and tremor. Excessive salivation was also part of the syndrome. A set of pediatric toxidromes is provided in the subsections that follow (Koren, 2007).

### Respiratory

- Difficulty breathing: Neuromuscular blockade is achieved by botulinum toxin; neuromuscular blockers such as d-tubocurarine, organophosphates, and carbamates (AChE inhibition at high doses with tremor, muscle weakness, agitation, seizures, and coma, but at lower doses induce vomiting, diarrhea, abdominal cramping, bradycardia, miosis, drooling, respiratory hypersecretion, and diaphoresis); strychnine; and tetanus. CNS depression occurs with opiates, ethanol (or other alcohols), sedative-hypnotics, and tricyclic antidepressants. Other factors that may lead to labored breathing are carbon monoxide, cyanide, metabolic acidosis, liver failure, heart failure, methemoglobinemia, and salicylates.
- Hypoxia: carbon monoxide poisoning, nitrate poisoning or other methemoglobinemia, hemolysis caused by glucose-6-phosphate dehydrogenase (G-6PDH) deficiency (favism), cyanide poisoning.
- Wheezing: beta-antagonists, irritant gases such as chlorine, hydrocarbons, isocyanates, organophosphates, carbamates, smoke inhalation, foodborne sulfites.

### Changes in Osmolarity and Ion Balance

- Osmolarity gap: Because osmolarity should be  $290 \text{ mOsm/kg} (= 2 \times [\text{Na}^+ (\text{mEq/L})] + \text{glucose} [\text{mg/dL}]/18 + \text{blood urea nitrogen} [\text{mg/dL}]/2.8)$ , alterations in osmolarity may be caused by acetone, ethanol, ethyl ether, ethylene glycol, isopropyl alcohol, mannitol, methanol, renal failure, and ketoacidosis (type I diabetic and alcoholic).
- Anion gap: Calculated as  $\text{Na}^+ - ([\text{Cl}^-] + [\text{HCO}_3^-])$ , where normal ranges from 8–12

mEq/L, this may be caused by acetaminophen overdose (as would liver failure and jaundice), beta-adrenergic medications, carbon monoxide, cyanide, iron, isoniazid, salicylates, theophylline, toxic alcohols, and the antiseizure medication valproic acid.

### Neuromuscular

- Acute movement disorder: Dystonia (twisting and repetitive movements) may be caused antipsychotics and metoclopramide. Dyskinesia (difficulty with/distortion of voluntary movements as in tic, chorea, spasm, or myoclonus) may be produced by amphetamines, anticholinergics, antihistamines, cocaine, gamma-hydroxybutyrate, selective serotonin reuptake inhibitors (SSRIs), and tricyclic antidepressants. Rigidity may be caused by malignant hyperthermia (dangerous increase in temperature that is a reaction to general anesthetic agents and succinylcholine), neuroleptic malignant syndrome, and phencyclidine.
- Coma: Alcohols, anticholinergics, arsenic, beta-receptor antagonists, cholinergic agents, carbon monoxide, lead, lithium, opioids, phencyclidine, phenothiazine antipsychotics, salicylates, sedative-hypnotics, and tricyclic antidepressants.
- Opioid overdose: CNS depression (sedation and lethargy to coma), respiratory depression, hypoxia, and miosis (no pupil constriction or miosis with other sedative-hypnotic agents). Miosis may also be caused by cholinergic agents, clonidine (adrenergic alpha-2 receptor agonist that decreases sympathetic outflow from the CNS), nicotine, phenothiazines, and phencyclidine (PCP). The opposite of miosis is dilation or mydriasis caused by anticholinergics (belladonna alkaloids), glutethimide, meperidine, sympathomimetics (mimic action of norepinephrine and epinephrine), and withdrawal from CNS depressants such as alcohol or opiates.
- Seizures: Amphetamines, cocaine, caffeine, theophylline, tricyclic antidepressants, venlafaxine, phenothiazines and butyrophenones,

camphor, organophosphates, carbamates, ethylene glycol, isoniazid, meperidine, methanol, salicylates and any withdrawal from psychoactive drug (likely depressants—see withdrawal symptoms).

- Serotonin syndrome (hot and confused): Monoamine oxidase inhibitors (MAOIs) in combination with SSRIs lead to akathisia-like restlessness, muscle twitches, myoclonus, hyperreflexia, diaphoresis (excess perspiration), penile erection, shivering, tremor, and with increased severity seizures and coma. Other agents that can increase temperature are anticholinergics (accompanied by dry flushed skin, dry mouth, mydriasis, delirium, hallucinations, tachycardia, ileus or decreased peristalsis, urinary retention, and in most severe poisonings coma and respiratory arrest), MAOIs, metals, phencyclidine, phenothiazines (accompanied by muscle rigidity, metabolic acidosis, and confusion for neuroleptic overdose), salicylates, sympathomimetics, and withdrawal from CNS depressants such as ethanol and opioids. Decreased temperature or hypothermia can be caused by beta-blockers, carbon monoxide, cholinergic agonists, ethanol, hypoglycemia-inducing agents, and sedative-hypnotics.
- Sleepiness: Antihistamines, any sedative-hypnotic, alcohols, gamma-hydroxybutyrate, tricyclic antidepressants, opioids, carbon monoxide, cyanide, and hypoglycemic agents.
- Withdrawal symptoms: Withdrawal from depressants or toxicity from high levels of stimulants leads to diaphoresis, diarrhea, fever, insomnia, hypertonia, hyperreflexia, lacrimation, respiratory distress, seizures, and tachycardia. Withdrawal from stimulants has the opposite effects such as bradycardia, depression, increased appetite (nicotine withdrawal), and sleepiness.

### Cardiovascular

- Blood pressure:
  - Hypertension: Glycyrrhizin, mineralocorticoids, agents that may cause tachycardia, alpha-1 agonists such as

phenylephrine, and cadmium or other heavy or metals (or other toxicants) that induce renal damage or lead to adrenal tumors.

- Hypotension: Carbon monoxide, cyanide, hemorrhagic agents, iron, opioids, nitrites, phenothiazines, sedative-hypnotics, tricyclic antidepressants, and theophylline.
- Cardiac:
  - Bradycardia (too slow): Beta-receptor antagonists (especially beta-1 blockers), calcium-channel blockers, cardiac glycosides (e.g., digitalis), clonidine, cyanide, lithium, nicotine, opioids, carbamates and organophosphates (AChE inhibitors), and parasympathomimetics (mimics action of acetylcholine).
  - (Atrial) tachycardia (too fast): Amphetamines, antihistamines, caffeine, clonidine, cocaine, theophylline, carbon monoxide, cyanide, hydrogen sulfide, anticholinergics (antihistamines, phenothiazines, tricyclics and atropine), ethanol, phencyclidine, sympathomimetics, withdrawal from any depressive psychoactive drug, theophylline, and thyroid hormone.
  - Ventricular arrhythmias: Speeding from amphetamines, cocaine, caffeine, chloral hydrate, aromatic hydrocarbons, anticholinergics and theophylline. Q-T prolongation that can lead to torsade de pointes (polymorphic ventricular tachycardia that may become deadly ventricular fibrillation) may be caused by the antiarrhythmic medication amiodarone, arsenic, chloroquine, quinine, quinidine, organophosphates, and tricyclic antidepressants.

### Hepatic

- Acetaldehyde (metabolite of ethanol), acetaminophen, aflatoxin, *Amanita phalloides* (death cap mushroom) or similar species, carbon tetrachloride, other chlorinated hydrocarbons that at lower doses may also lead to kidney failure, halothane, phenol, phosphorus, and valproic acid.

## Renal

- Direct: Glomerulonephritis can be caused by mercury and gold compounds as indicated by proteinuria (Bigazzi, 1994). Heavy metal exposure as assessed by cadmium and other metals found in the urine appear to cause early kidney damage as indicated by an increase in urinary *N*-acetyl- $\beta$ -D-glucosaminidase (Thomas et al., 2009).
- Indirect: Statins have caused rhabdomyolysis (muscle wasting) and ensuing renal failure (Omar et al., 2001).

## Skin

- Stevens-Johnson syndrome and toxic epidermal necrolysis: Sulfonamides, aromatic antiepileptics, lamotrigine, penicillins, doxycycline, and nevirapine. Other skin problems can be found with strong acids and bases (chemical burns), anilines (darkening), carbon monoxide (cherry red coloration), cyanide (blue under finger nails, but if skin exposed may find a redness and a bitter almond-like smell), strychnine (dark face and neck), metals (certain metals give a metallic luster to the skin), vanadium (green tongue).

## Toxicoproteomics

A new field worth examining is toxicoproteomics. Using model liver and kidney toxicants, biomarkers for organ-specific toxicity are being determined (Merrick and Witzmann, 2009). Samples can be subjected to two-dimensional gel electrophoresis and mass spectrometry, liquid chromatography of protein digests that are analyzed by tandem mass spectrometers, retentate chromatography-mass spectrometry (laser-based mass spectrum), and antibody arrays available for toxicoproteomic studies. Analysis of the liver must involve both liver cells and the plasma, because injured organs release peptides and proteins into the plasma. In the case of the liver, some of the plasma proteins are normally synthesized by the liver. Liver toxicants such as acetaminophen, bromobenzene, dichlorobenzenes, lipopolysaccharide,

and monocrotaline serve as model agents for causing hepatic necrosis and inflammation. Male rats are treated with a vehicle as a control, a low dose to observe effects not expected to be associated with injury, and a high dose to cause injury. Rats are sampled at 6, 24, and 48 hours following administration of the toxic agent. These times are associated with changes in histopathology and serum chemistries (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) and account for the time necessary to overwhelm the reduced glutathione content from reactive metabolites. Five levels of analysis are possible by this approach. First is matching gene alterations detected by DNA microarrays with altered protein profiles. Second is to compare affected pathways through altered gene transcripts and proteins responsible for activation or repression events. Third is analysis of posttranslationally altered proteins as they are related to modified genes encoding for kinases, proteases, phosphorylases, conjugation enzymes, or others that facilitate those post-translational processes. Fourth is a proteomic determination of alterations of protein trafficking and signaling pathways or subcellular site(s) of injury in nuclei, mitochondria, endoplasmic reticulum, plasma membrane, and cytosol. Fifth is the kinetic protein analysis of the changes in the serum proteome over the pretoxic, toxic, and recovery periods (Merrick, 2006).

## Sampling Techniques

Once a presumed diagnosis has been made, the sampling should be made easier unless the toxicity proceeds to lethality unnoticed and with no clear indication of cause. In that case, an autopsy is performed. A living being is always preferred, as death results in tissue destruction, possible loss of toxicant, and redistribution of toxicant. For instance, if the time of death is known, the amount of cyanide may be extrapolated back to the time of death based on the length of time the sample remained in the cadaver, length of storage time of the sample, preservation of the sample by addition of sodium fluoride, and storage conditions of the sample (McAllister et al., 2008). For example, antemortem and postmortem collection of

vitreous humor, femoral vein and artery, left and right heart ventricles indicated that pigs given a high intravenous (IV) dose of morphine had significantly different free and total morphine values before and after death and by site of injection over time. Free morphine concentrations were higher in postmortem samples, but total morphine was similar to slightly lower in postmortem samples. Antemortem and postmortem values are not consistent based on IV dose. The femoral vein had the lowest values in either case, which is consistent with the site of metabolism (Crandall et al., 2006). In another example, two antipsychotic drugs, haloperidol and thioridazine, administered intraperitoneally redistribute from the rat lung to the blood following death as indicated by liquid chromatography–tandem mass spectrometry. This is important, because fatal arrhythmias may result from a high dose of these antipsychotics (Castaing et al., 2006). A variety of pre- and postmortem examinations revealed a variable redistribution of psychiatric medications into the blood (Rodda and Drummer, 2006). Although earlier reports (1970s) of redistribution following death are available in the literature, at least one publication indicates many of the difficulties of obtaining reliable samples in a critically ill poisoned patient (Boyle et al., 2009). The problems of the nature of the poison or poisons—gas, volatile liquid, corrosive chemical, metals, anions and nonmetals, nonvolatile organic substances (organic strong acids, organic weak acids, organic bases, organic neutral chemicals, organic amphoteric compounds), or mixtures of compounds that may react with each other or complex or disperse into living or dead tissue—is one factor. A second problem is associated with sample collection, transport, and storage, where little may be known of the toxic agent. The site where the victim is found may be remote or not ideally suited for sample collection or storage (e.g., battlefield conditions, car accident, fire, explosion). The analytical methodology employed may not be optimal based on the facilities available in that part of the country or world. The circumstances of the exposure may involve how the poison was encountered and for how long (multiple routes of exposure

possible and many time sequences). Mechanical trauma or inhalation of stomach contents may complicate analysis (e.g., gunshot wounds, car accident). Tolerance or synergy due to prior or concurrent exposure may yield results that are initially not consistent with death (too low) or inconsistent with life (too high, especially in postmortem concentrations). For this reason, it is best to take as many fluids and tissues as possible postmortem, including peripheral blood, aqueous humors, urine, stomach contents, and so forth. Original compounds and metabolites will also give some indication of the total picture. As an example of the problems associated with sample collection and reliability, ethanol intoxication is a common poisoning in the population. Blood ethanol can be lost or produced especially in the presence of significant trauma, which happens often in alcohol-related deaths (Flanagan and Connally, 2005). What happens if only skeletal remains are found or the blood has been lost? One more recent publication indicates the possibility of bone as an indication of the presence of a medication or toxicant. The concentrations of the medications tested were generally higher in the bone than in the blood and some were not found in the bone. Thus, interpretation should be done cautiously. The encouraging factor is that there is at least one more possibility for the analyst when considering forensic determination of possible causes of death (McGrath and Jenkins, 2009). Sometimes the autopsy findings provide a toxicity not indicated previously by animal data. Two suicides by acetaminophen yielded rapid deaths without the usual hepatic centrilobular necrosis. Cardiac toxicity may cause death at high concentrations of acetaminophen in humans (Singer et al., 2007).

### Live for Human Performance

The Society of Forensic Toxicologists (SOFT) and American Academy of Forensic Sciences (AAFS) (2006) produced a document titled “Forensic Toxicology Laboratory Guidelines,” which describes in detail certified laboratory work in forensic toxicology including definitions, personnel, standard operating

procedures, samples and receiving, security and chain of custody, analytical procedures, quality assurance and quality control, review of data, reporting of results, interpretation of toxicology results, and safety. This section focuses on the sampling portion. SOFT and AAFS recommend taking 15 mL of blood for ethanol and other toxicants that are needed to assess performance. The “reasonable complete” drug screen should only require 5 mL or less of blood. That requires sensitive and specific methodology development by forensic toxicology laboratories. Urine should have a minimum collection volume of 30 mL for analysis for detection of metabolites or the last tissue to indicate the presence of an illegal agent. Hair samples may be taken for exposure to drugs of abuse, but laboratory ability to analyze should meet the Society of Hair Testing proficiency for detection without false positives or negatives following extraction in hydrochloric acid, enzymatic treatment (Pronase followed by liquid-liquid extraction, or  $\beta$ -glucuronidase treatment and buffer extraction), hot methanol extraction (with or without sonication), and hot NaOH disintegration and extraction (Jurado and Sachs, 2003). Urine, blood, and hair have been used for heavy metals and especially arsenic analysis. The fingernails prove to indicate the chronic occupational exposure of workers to arsenic (Agahian et al., 1990). Lead concentrations in teeth have been studied since the 1970s and still serve to monitor environmental exposures in children in foreign countries (Arruda-Neto et al., 2009).

### Postmortem

SOFT and AAFS (2006) recommend taking 50 grams of brain, liver, and kidney; 25 mL of heart blood; 10 mL of peripheral blood; and all available of the vitreous humor, bile, urine, and gastric contents as a start for all postmortem toxicology analyses. For specific toxicants lung, intestine, or other specimens may be taken. If a corpse has been deteriorating, even the organisms that are assisting in its decomposition can be sampled as was done for arthropod larvae *Chrysomya megacephala* (Fabricius) and

*Chrysomya rufifacies* (Macquart) in a suspected case of malathion poisoning (Gunatilake and Goff, 1989).

## Analysis Methodology

### Classical Analytical Chemistry Approach

The classical approach involves making an acid or base digest of the tissue. The acidified portion may be subject to steam to release the volatile medications and poisons such as acetaldehyde, acetone, carbon monoxide, cyanide, ethanol, isopropanol, methanol, phenol, and so forth or anions such as bromide, borate, fluoride, and oxalate. The acid fraction leaches metals out of the tissues and can be analyzed by atomic absorption or inductively coupled plasma emission spectroscopy. The basic extract is separated into an aqueous and an organic phase. The aqueous phase is acidified and organic solvent is added. This yields the acidic medications such as the barbiturates (from barbituric acid). This step can be preceded by a precipitation of protein or similar cleanup procedure. The organic phase of the organic extract is extracted with acid and again separated into an aqueous and organic phase. The organic phase contains the neutral medications such as simvastatin. The aqueous phase is made basic and contains the basic medications such as amphetamine (the amine portion of the catecholamine structure produces its basic nature). In the past, volatiles were analyzed by gas-liquid chromatography. The medications or street drugs were determined by thin-layer chromatography techniques. Organic extracts of the tissues would also be used to analyze for hydrophobic compounds such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and dioxins. If a chemical powder or unknown liquid is found near a corpse or stomach or intestinal contents yield a liquid, pill, or compound, they may be subjected to tests such as boiling point, melting point, and other physical determinations. These preparations may have a chemical added to it to form crystals. Chemical spot tests will be discussed later under rapid screening tests. Chromatography is the next

topic, as it is the method of choice if specificity is required. The World Health Organization (2015) recommends that analytical toxicology laboratories contain the following equipment and have procedures to perform simple “spot” tests: Conway apparatus, Gutzeit apparatus, direct-reading spectrophotometer, ultraviolet (UV)/visible recording spectrophotometer, thin-layer chromatography—qualitative, thin-layer chromatography—quantitative, gas chromatography—packed columns, gas chromatography—capillary columns, gas chromatography—flame ionization detection, gas chromatography—nitrogen-phosphorus detection, gas chromatography—electron-capture detection, gas chromatography—mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC)—UV detection, high-performance liquid chromatography—fluorescence detection, high-performance liquid chromatography—mass spectrometry (LC-MS), high-performance liquid chromatography—electrochemical detection, high-performance liquid chromatography—diode array UV detection, capillary electrophoresis, atomic emission spectrometry, atomic absorption spectrometry (flame), electrothermal atomic absorption spectrometry, inductively coupled plasma source spectrometry, radioimmunoassay—counting, enzyme immunoassay (e.g., enzyme-multiplied immunoassay technique), fluorescence immunoassay, enzyme-linked immunosorbent assay, fluorimetry, and infrared spectrometry. Pure standards are also required of the laboratory. Many of these techniques alone or in combination would not be trusted to identify an unknown. For example, SOFT/AAFS (2006) guidelines indicate that an identification by immunoassay followed by “confirmation” by flame ionization detectors and nitrogen-phosphorus detectors only give a range of suspects based on retention time of the columns and will not hold up in a court of law. Mass spectrometry (MS) gives structural features that may lead to identification of the unknown toxicant. The beam intensity for MS may be varied to change the amount of fragmentation of the compound, and low intensities may yield more of the molecular ion, which is the organic molecule with a positive charge due to removal of one electron.

### **Modern Extraction Linked to GC-MS, LC-MS, or LC-(Tandem) MS(/MS)**

The gold standard for toxicology analyses currently is an extraction procedure followed by GC-MS analysis. Not all toxicants are volatile for gas chromatographic analysis, and newer methods employ liquid chromatography coupled with one or two mass spectrometers (Maurer, 2007). Because the sample matrix may be complicated with a variety of contaminants in the extract, mass spectra can now have a standardized computer program to subtract out interfering substances and provide evidence of the structure for unknown toxicants not available in standard MS libraries (Stimpfl et al., 2003). In June 1986, a poisoning epidemic in Sierra Leone was solved employing positive chemical ionization mass spectrometry and nuclear magnetic resonance spectroscopy. An unknown poison in bread samples proved to be the organophosphorus pesticide parathion (Hill et al., 1990). Note that two different methods of analysis were used as is recommended for determination and confirmation of an unknown toxicant. The development of LC-MS(/MS) has led to determination of unknown toxicants, and with the proper algorithms (e.g., SALSA [Stochastic Approach for Link-Structure Analysis]) it can yield epoxide adducts of proteins. Hemoglobin adducts are likely, such as those formed on incubation with styrene oxide, ethylene oxide, and butadiene oxide (Badghisi and Liebler, 2002). The biological sample in this case represents the aqueous phase and the organic solvent the second phase that involve high-throughput liquid–liquid extraction (LLE) methodology by robotic liquid handling of a 96-well LLE plate with inert diatomaceous earth particles for continuous and efficient extraction of analytes between the aqueous biological sample and the organic extraction solvent (Peng et al., 2001).

### **Rapid Screening Tests**

The older color tests are used heavily by the law enforcement agencies for trafficking in illegal drugs. The U.S. Department of Justice, National Institute of Justice has a Standards and Testing

Program that has developed a list of Color Test Reagents/Kits for Preliminary Identification of Drugs of Abuse (NIJ Standard-0604.01), displayed in **Table 14-1**. There are also commercially available test kits for urine that measure 12 standard abused medications or street drugs and their metabolites using simple immunoassays to lateral flow-based immunoassays. For example, one commercially available kit uses sensitivities recommended by the National Institute on Drug Abuse to screen urine for the following compounds and their metabolites in urine: 25 ng/mL for phencyclidine, 50 ng/mL for marijuana (THC); 100 ng/mL for oxycodone, 300 ng/mL for cocaine, methadone, barbiturates, benzodiazepine, or propoxyphene; 500 mg/mL for MDMA (ecstasy), 1,000 mg/mL for amphetamine or methamphetamine; and 2,000 mg/mL for opiates.

Detection time is important in living beings. Peptide hormones are undetectable in urine. Cocaine detection may range from 1–4 days following administration. Propoxyphene can be detected from 6 hours to 2 days after use. Short-acting barbiturates may be detectable for

2 days. Amphetamines may be detected from 2–5 days after use. Clenbuterol can be found in urine 4–6 days following use. Nicotine may be observed in urine 4–10 days after a person ceases smoking. A 5–7 day detection time is common to euphorics such as ecstasy, the opiates, the dissociative anesthetic agent ketamine, and methamphetamines. Cannabinoids such as THC are detectable in urine for 5 days after one-time use to as high as 48–63 days in those using marijuana daily. Benzodiazepines may be detected in urine from 7–10 days following cessation of use. Oral anabolic steroids may be detected for 14–28 days, while injected steroids are detectable 1–3 months following use. People have employed diuretics to decrease the detectable concentration of the medication, but a specific gravity test and a test for diuretics indicate that a person is trying to avoid detection of a banned substance or indicate that use was below the legal limit for operating a motor vehicle or practicing medicine. Specific gravity is also altered by salts or detergents. Other compounds that may be attempted to be added to urine to avoid detection of banned substances or abused drugs

**TABLE 14-1** Color Changes for Medications and Drug of Abuse Tests

Reagent (#)	Color Change	Drugs (form or solvent)
Cobalt Thiocyanate (A.1)	Brilliant greenish blue	Benzphetamine HCl, brompheniramine maleate, chlordiazepoxide HCl, chlorpromazine HCl, doxepin HCl, hydrocodone tartrate, methadone HCl, methylphenidate HCl
	Strong greenish blue	Cocaine HCl, diacetylmorphine HCl, ephedrine HCl, meperidine HCl, phencyclidine HCl, procaine HCl, propoxyphene HCl, pseudoephedrine HCl
	Strong blue	Quinine HCl (all in CHCl <sub>3</sub> )
Dille-Koppanyi Reagent. Modified (A.2)	Light purple	Amobarbital, pentobarbital, phenobarbital, secobarbital (all in CHCl <sub>3</sub> )
Duquenois-Levine Reagent. Modified (A.3)	Strong reddish purple to very light purple	Mace (crystals)
	Pale reddish purple to light gray purplish red	Nutmeg (extract)
	Light yellow green	Tea (extract)
	Gray purplish blue to light purplish blue to deep purple	Tetrahydrocannabinol, THC (in ethanol)

(continues)

**TABLE 14-1** Color Changes for Medications and Drug of Abuse Tests (*continued*)

Reagent (#)	Color Change	Drugs (form or solvent)
Mandelin Reagent (A.4)	Moderate olive Grayish olive green Brilliant yellow green Strong orange Dark olive  Deep orange yellow Strong yellow Moderate bluish green Dark yellowish green Moderate reddish brown Dark olive brown Dark reddish brown Grayish olive Moderate olive green Bluish black  Dark yellowish brown Dark grayish blue Very orange yellow Brilliant orange yellow Dark grayish reddish brown Dark brown Dark greenish yellow Deep orange Deep greenish yellow	Acetaminophen (CHCl <sub>3</sub> ) Aspirin (powder) Benzphetamine HCl (CHCl <sub>3</sub> ) Brompheniramine maleate (CHCl <sub>3</sub> ), salt (crystals) Chlorpromazine HCl, codeine (both in CHCl <sub>3</sub> ), Excedrin (powder)  Cocaine HCl (CHCl <sub>3</sub> ) Contac (powder) d-Amphetamine HCl (CHCl <sub>3</sub> ) d-Methamphetamine HCl (CHCl <sub>3</sub> ) Diacetylmorphine HCl (CHCl <sub>3</sub> ) Dimethoxy-meth HCl (CHCl <sub>3</sub> ) Doxepin HCl, propoxyphene HCl (both in CHCl <sub>3</sub> ) Dristan (powder) Mace (crystals) d,l-3,4-Methylenedioxyamphetamine HCl, MDA (CHCl <sub>3</sub> )  Mescaline HCl (CHCl <sub>3</sub> ) Methadone HCl (CHCl <sub>3</sub> ) Methaqualone (CHCl <sub>3</sub> ) Methylphenidate HCl (CHCl <sub>3</sub> ) Morphine monohydrate (CHCl <sub>3</sub> ) Opium (CHCl <sub>3</sub> ) Oxycodone HCl (CHCl <sub>3</sub> ) Procaine HCl (CHCl <sub>3</sub> ) Quinine HCl (CHCl <sub>3</sub> )
Marquis Reagent (A.5)	Deep red Deep reddish brown Deep purplish red Very dark purple Strong reddish orange Deep reddish orange Dark reddish brown  Moderate orange Blackish red Dark grayish red Dark red Olive black Moderate yellow Dark reddish brown	Aspirin (powder) Benzphetamine HCl (CHCl <sub>3</sub> ) Chlorpromazine HCl, diacetylmorphine HCl (both in CHCl <sub>3</sub> )  Codeine (CHCl <sub>3</sub> ) d-Amphetamine HCl (CHCl <sub>3</sub> ) d-Methamphetamine HCl (CHCl <sub>3</sub> ) d-Amphetamine HCl, d-Methamphetamine HCl (both in CHCl <sub>3</sub> )  Dimethoxy-meth HCl (CHCl <sub>3</sub> ) Doxepin HCl (CHCl <sub>3</sub> ) Dristan (powder) Excedrin (powder) Lysergic acid diethylamide. LSD (CHCl <sub>3</sub> ) Mace (crystals) Dristan (powder)

*(continues)*

**TABLE 14-1** Color Changes for Medications and Drug of Abuse Tests (*continued*)

Reagent (#)	Color Change	Drugs (form or solvent)
Marquis Reagent (A.5) ( <i>continued</i> )	Black Deep brown Strong orange Light yellowish pink Moderate orange yellow Very deep reddish purple Dark grayish reddish Brown Pale violet Blackish purple Dark brown	MDA (CHCl <sub>3</sub> ) Meperidine HCl (CHCl <sub>3</sub> ) Mescaline HCl (CHCl <sub>3</sub> ) Methadone HCl (CHCl <sub>3</sub> ) Methylphenidate HCl (CHCl <sub>3</sub> ) Morphine monohydrate (CHCl <sub>3</sub> ) Opium (powder)  Oxycodone HCl (CHCl <sub>3</sub> ) Propoxyphene HCl (CHCl <sub>3</sub> ) Sugar (crystals)
Nitric Acid (A.6)	Brilliant orange yellow  Light greenish yellow Pale yellow Very yellow Brilliant yellow Deep orange Strong brown Moderate greenish yellow Dark red Dark orange yellow Brilliant yellow	Acetaminophen (CHCl <sub>3</sub> ), Excedrin (powder), Morphine monohydrate (CHCl <sub>3</sub> ) Codeine, MDA (both in CHCl <sub>3</sub> ) Diacetylmorphine HCl (CHCl <sub>3</sub> ) Dimethoxy-meth HCl (CHCl <sub>3</sub> ) Doxepin HCl (CHCl <sub>3</sub> ) Dristan (powder) Lysergic acid diethylamide (LSD) (CHCl <sub>3</sub> ) Mace (crystals) Mescaline HCl (CHCl <sub>3</sub> ) Opium (powder) Oxycodone HCl (CHCl <sub>3</sub> )
<i>Para</i> -Dimethylamino-Benzaldehyde (A.7)	Deep purple	LSD (CHCl <sub>3</sub> )
Ferric Chloride (A.8)	Dark greenish yellow Deep orange Very orange Moderate purplish blue Dark green	Acetaminophen (methanol) Baking soda (powder) Chlorpromazine HCl (methanol) Dristan, Excedrin (both powder) Morphine monohydrate (methanol)
Froede Reagent (A.9)	Grayish purple Very deep red Very dark green Moderate olive brown Deep purplish red Very yellow green Deep reddish brown Light bluish green	Aspirin (powder) Chlorpromazine HCl (CHCl <sub>3</sub> ) Codeine (CHCl <sub>3</sub> ) Contac (powder) Diacetylmorphine HCl, morphine monohydrate (both in CHCl <sub>3</sub> ) Dimethoxy-meth HCl (CHCl <sub>3</sub> ) Doxepin HCl (CHCl <sub>3</sub> ) Dristan (powder)

*(continues)*

**TABLE 14-1** Color Changes for Medications and Drug of Abuse Tests (*continued*)

Reagent (#)	Color Change	Drugs (form or solvent)
Froede Reagent (A.9) ( <i>continued</i> )	Brilliant blue Moderate yellow green Light olive yellow Greenish black Brownish black Strong yellow Dark grayish red Brilliant yellow	Excedrin (powder) LSD (CHCl <sub>3</sub> ) Mace (crystals) MDA HCl (CHCl <sub>3</sub> ) Opium (powder) Oxycodone HCl (CHCl <sub>3</sub> ) Propoxyphene HCl (CHCl <sub>3</sub> ) Sugar crystals
Mecke Reagent (A.10)	Blackish red Very dark bluish green Moderate olive brown Deep bluish green Dark brown Very dark red Light olive brown Dark grayish yellow Dark bluish green Greenish black Dark grayish olive Moderate olive Brownish black Olive black Deep reddish brown Brilliant greenish yellow	Chlorpromazine HCl (CHCl <sub>3</sub> ) Codeine, MDA HCl, morphine monohydrate (all in CHCl <sub>3</sub> ) Contac (powder) Diacetylmorphine HCl (CHCl <sub>3</sub> ) Dimethoxy-meth HCl (CHCl <sub>3</sub> ) Doxepin HCl (CHCl <sub>3</sub> ) Dristan (powder) Excedrin (powder) Hydrocodone tartrate Excedrin (CHCl <sub>3</sub> ) LSD (CHCl <sub>3</sub> ) Mace (crystals) Mescaline HCl, Oxycodone HCl (both in CHCl <sub>3</sub> ) Nutmeg (leaves) Opium (powder) Propoxyphene HCl (CHCl <sub>3</sub> ) Sugar (crystals)
Zwicker Reagent (A.11)	Light blue Light green Light purple Moderate yellow green Moderate yellowish green	Baking soda (powder) Excedrin (powder) Pentobarbital, phenobarbital, secobarbital (all in CHCl <sub>3</sub> ) Tea (leaves) Tobacco (leaves)
Simon's Reagent (A.12)	Dark blue Deep blue Pale violet	d-Amphetamine HCl, MDMA HCl (both in CHCl <sub>3</sub> ) Dimethoxy-meth HCl (CHCl <sub>3</sub> ) Methylphenidate HCl (CHCl <sub>3</sub> )
<p>Modified from National Institute of Justice Law Enforcement and Corrections Standards and Testing Program. 2000. Color test reagents/kits for preliminary identification of drugs of abuse (NIJ Standard 0604.01). Washington, DC: U.S. Department of Justice, Office of Justice Programs, National Institute of Justice. <a href="http://www.ncjrs.gov/pdffiles1/nij/183258.pdf">http://www.ncjrs.gov/pdffiles1/nij/183258.pdf</a></p>		

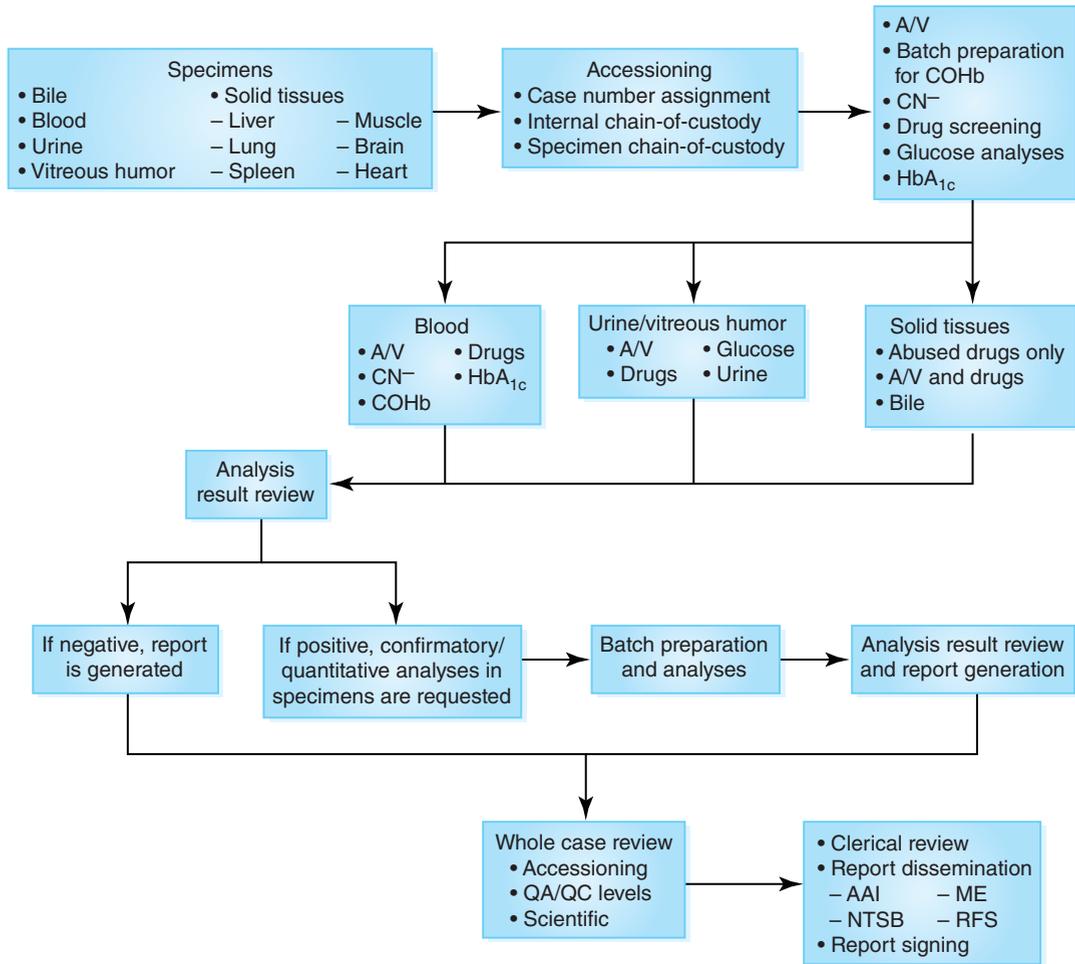
are acids and baking soda (change pH and can be detected by pH as well) and bleach to oxidize compounds or test components (odor and color test can detect these). If urine tampering is suspected, human physiological values of urine temperature (32.2–37.8°C), pH (4.5–8.0), and creatinine (> 20mg/dL) should be found. If urinary creatinine is < 20 mg/dL, it is diluted urine. Below 5 mg/dL creatinine, the sample is unlikely to be human urine. Specific gravity should be  $\geq 1.003$  g/mL and human IgG should be  $\geq 0.5$  mg/L. Some adulterants commonly used are glutaraldehyde (fixative that interferes with immunoassays and can be detected by gas chromatography), nitrite (oxidizing agent that can be detected colorimetrically), and pyridinium chlorochromate (oxidizing agent that can be detected by color, atomic absorption, or gas chromatography; Jaffee et al., 2008). Some “blocking agents” are more mythology than effective. For example, probenecid is a medication that decreases penicillin excretion. It does not work to retard anabolic steroid excretion and is also a banned substance in athletic competition even if it could do so. It is more difficult to alter professionally drawn blood samples, which are mostly useful for detection of toxicants and essential for therapeutic drug monitoring. Hair samples have increased longevity for substances and may detect use of drugs such as amphetamines, marijuana, cocaine, opiates, and phencyclidine for as long as 90 days following exposure. The combination of presence of a substance, its concentration at a given time following use, and its redistribution following death (determining minimum body burden may be more important in this case) is important, because the presence may indicate abuse, and excess leading to death may determine that a homicide was committed. This is especially true for injected substances that can only be legally obtained and administered by a medical practitioner. The investigation of the death of a very famous pop star headed in that direction as the number of medications and their concentrations were determined following autopsy (physician convicted of involuntary manslaughter due to administration of a fatal dose of the anesthetic agent propofol).

### Confirmatory Tests

Confirmatory test usually require greater sensitivity and usually employ GC-MS or LC-MS as the gold standard for identification. For example, confirmatory tests require the sensitivity for marijuana at 15 ng/mL, cocaine at 150 ng/mL, and amphetamines at 500 ng/mL. However, phencyclidine and opiates remain at the screening values of 25 ng/mL and 2,000 ng/L, respectively.

### Flow Chart of a Typical Postmortem Examination

An example where postmortems are common are crashes involving air transportation. The Federal Aviation Administration (FAA) has difficulty obtaining uniform samples from crashes, because explosions and other effects of impact may make recovery of certain biological fluids impossible. Note the flow chart in [Figure 14-1](#). It is clear that esoteric toxicants are not considered in the first analysis. Clearly, carbon monoxide poisoning is a primary concern when a fire occurs in an enclosed plane or in vehicles powered by a combustion engine as indicated by the test for carboxyhemoglobin (COHb). That is also a concern in northern climates. The state of Minnesota now requires dwellings to contain carbon monoxide detectors by bedrooms in houses with furnaces that use combustion for heating purposes or contain a gas stove. Cyanide is another toxicant that occurs during a fire, and it may occur naturally in certain plants and has been employed in poisonings that could account for a plane crash. Alcohol or other volatile substances may render the pilot less capable of handling an aircraft safely due to the CNS effects of volatile solvents. Additionally, other possibly abused street drugs or prescription medications are of general concern during investigation of the reason for a plane or other crash involving the U.S. Department of Transportation. Glucose is important, because hypoglycemia may account for unconsciousness. Additionally, uncontrolled diabetes mellitus is a concern for pilots as indicated

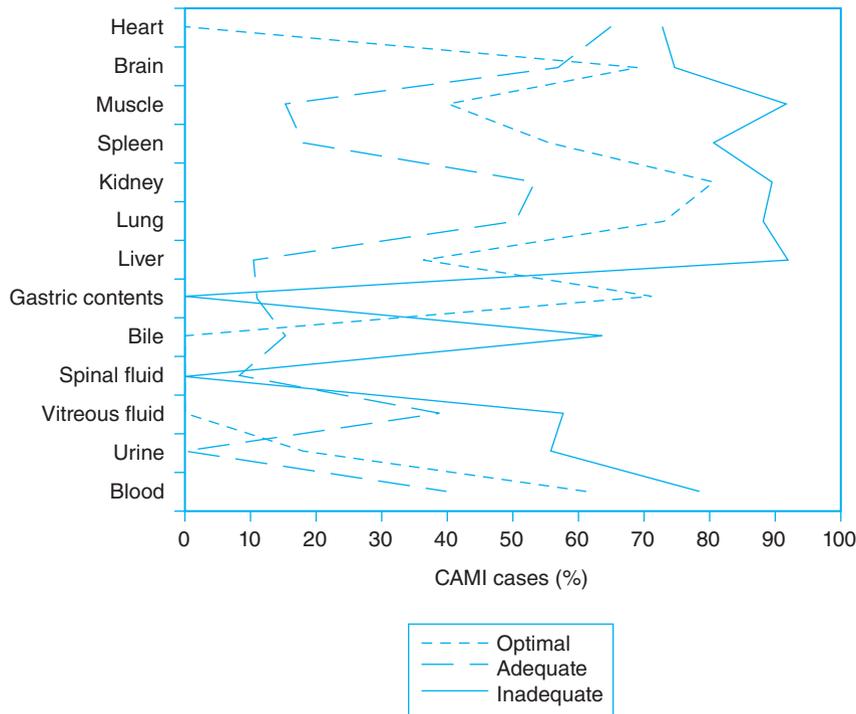


**FIGURE 14-1** A Flowchart for a Typical Toxicological Processing of Postmortem Biological Samples

<http://www.hf.faa.gov/docs/508/docs/cami/0214.pdf>

both by the glucose measurement and the use of the hemoglobin (Hb) A1c test which determines whether Hb is irreversibly glycosylated at the N-terminal valine of the  $\beta$ -chain (Nakanishi et al., 2003). The Civil Aerospace Medical Institute laboratory optimally requests 40 mL of blood in a green-top tube for COHb, blood  $\text{CN}^-$ ,  $\text{HbA}_{1c}$ , and drug analyses. The same amount of blood in a grey-top tube is used for ethanol and drug analyses. Urine (100 mL optimally) is collected for drug, ethanol, and glucose analyses. Vitreous humor (2 mL optimally) is sampled for ethanol and glucose testing. Bile (10 mL) is analyzed by radioimmunoassay

(RIA) when urine cannot be collected. Gastric contents (100 mL) are used as necessary for drug analyses. Ethanol and drug analyses are also performed on 500 g of liver; 300 g of skeletal muscle; 150 g of spleen; 100 g of lung, kidney, and brain; and 50 g of heart. Spinal fluid will be sampled for the amount available and tested for ethanol and perhaps drugs if enough is present. The problem is that all these tissues may not be available or only available at suboptimal (adequate or less) volumes. This is demonstrated in **Figure 14-2**. The kidneys are found at the most protected portion (most dorsal) of the abdominal area and therefore had



**FIGURE 14-2** Optimal, Adequate, and Inadequate Amounts of Various Postmortem Biological Specimens Received at CAMI from the 1,891 Cases (Fatalities) of the United States Fatal Aviation Accidents that Occurred During 1996–2000

<http://www.hf.faa.gov/docs/508/docs/cami/0214.pdf>

80% of the cases having optimal to adequate amounts of this tissue for examination for the years 1996–2000 (Chaturvedi et al., 2002). Uncontaminated spinal fluid is hardest to obtain, with less than 10% of the cases having optimal volumes for analyses. Urine is very useful in detecting metabolites of chemicals that may have already been excreted, but unfortunately appears to be inadequately available in more than 80% of the crashes. Blood is also heavily relied upon in toxicology for current levels of toxicants, the control of diabetes mellitus, and the clear indication of succumbing to fire (COHb and CN<sup>-</sup>). Unfortunately, optimal and adequate samples were only available in slightly more than 60% of the cases. It is interesting to note that gastric contents were more likely to be in optimal adequate volumes than blood. This is why it is important to consider all possible recoverable uncontaminated samples and what might be gained from their analyses.

## Therapeutic Drug (Medication) Monitoring in Living Human Patients

On occasion, certain drugs carry a high danger of toxicity (small therapeutic index or LD<sub>50</sub>/ED<sub>50</sub>) and generally are so lipophilic that they require extensive monitoring. Other reasons for following plasma concentrations are that the pharmacokinetics vary widely by patient, plasma concentration is a better measure than dose for therapeutic and adverse effects, there is a plasma concentration that must be achieved to ensure proper onset of therapy (and must be kept close to that value), or the therapeutic effects are difficult to monitor. The last two points are similar to the determination of the international normalized ratio (INR), which is used to determine anticoagulant therapy with a medication such

as warfarin (Anand and Yusuf, 2003). The target INR is 2–3. If the coagulation is too rapid, then dangerous clots may form. If coagulation is too low, hemorrhage may occur. This is specifically not therapeutic drug monitoring as the effect rather than the concentration of the medication is monitored. When effect is not well measured, then plasma concentration may be the next best option. Proper dosing is not possible without measuring resultant plasma concentrations. The journal *Therapeutic Drug Monitoring* is devoted to this area.

A number of factors must be considered regarding monitoring of medications. These include compliance (whether the patient is taking the medication as prescribed) and age, because neonates, children, and the elderly absorb, metabolize, and excrete differently than adults. The gender of the patient and pregnancy play important roles in pharmacokinetics/toxicokinetics. Hepatic disease may hamper metabolism. Renal disease can influence half-life and clearance. Cardiovascular disease can decrease or affect circulation and blood pressure, which is important regarding assumptions of speed of action, organ distribution, or filtration resulting in excretion. Respiratory disease affects circulation, blood pH, and is especially important in anesthetic gases and excretion of volatile components of the medication. Other medications clearly may interact in a toxic fashion with the medication or alter metabolism or elimination rate. Additionally, tolerance and dependence must be considered. There are a variety of environmental factors that influence drug metabolism. Genetic polymorphisms of drug metabolism also influence plasma concentrations. To do medication monitoring properly, the doctor takes a history that includes the patient's age, weight, sex, their genetic history of disease, the nature of their current disease, medications taken, doses, medication schedule, and for the specific medication(s) for which plasma concentrations must be determined the time of last dose, time of specimen, and the clinical status relevant to the drug used (e.g., blood pressure, ECG, EEG, plasma liver enzymes). Consider [Table 14-2](#), where therapeutic ranges are reported for a variety of medications. The lowest value is the trough concentration ( $C_{\text{trough}}$ )

below which no therapeutic value is indicated for the medication alone. This trough concentration works well for antibiotics and antivirals, as the  $C_{\text{trough}}$  for the HIV medication atazanavir, for example, is associated with efficacy (de Requena et al., 2005). Other medications may indeed change this value significantly, as is the case for certain combinations of protease inhibitors used to treat HIV infection (Guiard-Schmid et al., 2003). For anti-epileptic medications, the plasma concentration of lamotrigine varies four-fold when comparing joint anti-seizure therapy with the metabolic enzyme inhibitor valproic acid as opposed to inducers phenytoin or carbamazepine (Morris et al., 1998). The high peak concentration should represent a toxic threshold of sorts that would endanger the patient and have less therapeutic value. Note that for the cancer chemotherapy agent methotrexate, only the trough may be relevant. This indicates that all concentrations that are therapeutic are toxic as well, but hopefully more so to cancer cells. Toxicity to normal tissues is to be monitored carefully, so this may obviate the need for a peak value. Some compounds have controversial values or published articles indicating the lack of need for therapeutic drug monitoring for certain medications. Rapid metabolism of the medication may lead to a difficult match between therapeutic effect and plasma concentration of the unaltered drug. This is true for the immunosuppressant purine anti-metabolite azathioprine (Lin et al., 1980). Also, binding to the plasma protein albumin in the blood makes the compound unavailable for activity. Certain compounds such as the anti-seizure medication valproic acid and the anti-inflammatory salicylate have non-linear protein binding in their therapeutic range (Birkett, 1994). Other compounds have uncertain therapeutic value, such as may be the case for a person who has had HIV long enough to experience mutations in the virus that renders the protease inhibitor less effective. Some compounds may be well described by their area under the curve value, which requires many points and appears to be impractical. One such compound is the immunosuppressant mycophenolate mofetil, which is used in kidney transplant patients. The selection of a sampling time is difficult

**TABLE 14-2** Therapeutic Drug Monitoring Based on Class of Drugs, Concentration Range, Monitoring Method, and Interaction with Other Medications

Class of Medication	Generic Name	Therapeutic Plasma Concentration Range
Aminoglycoside antibiotics	Amikacin Gentamicin Tobramycin	15–25 µg/mL 5–10 µg/mL 5–10 µg/mL
Other toxic antibiotics	Chloramphenicol Vancomycin	10–20 µg/mL 5–10 µg/mL or 10–20 µg/mL trough (30 min prior to next dose = controversial)
Anti-arrhythmics	Amiodarone Digoxin Digitoxin Flecainide Lidocaine Mexiletine Procainamide N-Acetyl procainamide (metabolite) Quinidine	0.5–2.0 µg/mL 0.5–2.0 ng/mL 10–30 ng/mL 0.2–1.0 µg/mL 1.5–5.0 µg/mL 0.5–2.0 µg/mL 4.0–8.0 µg/mL 10–20 µg/mL 2.0–5.0 µg/mL
Anti-arthritis or anti-inflammatory	Salicylates	100–250 µg/mL
Antiepileptics	Carbamazepine Ethosuximide Gabapentin Lamotrigine Phenobarbital Phenytoin Valproic acid	5–12 µg/mL 40–100 µg/mL 2–10 µg/mL 3–14 µg/mL 10–30 µg/mL 10–20 µg/mL 50–100 µg/mL
Bronchodilators	Caffeine Theophylline	10–20 µg/mL 10–20 µg/mL
Cancer chemotherapy	Methotrexate	> 0.01 µmol/L
HIV treatments — protease inhibitors	Atazanavir Indinavir Lopinavir Nelfinavir Ritonavir Saquinavir	150–850 ng/mL 0.15 µg/mL trough, 10 µg/mL peak 3–8 µg/mL 0.7–1.0 µg/mL (trough) 1.1–6.3 µg/mL (trough) 100 ng/mL (trough minimum for wt virus)
Immunosuppressants	Azathioprine Cyclosporine Mofetil mycophenolate Sirolimus Tacrolimus	20–90 ng/mL at peak (mercaptopurine metabolite) C <sub>2</sub> (conc 2 hours after dosing) > 800 ng/dL 1–3.5 µg/mL (C <sub>2</sub> trough) 4–20 ng/mL (C <sub>2</sub> trough less on initiation, more for maintenance) 5–20 ng/mL
Psychiatric medications	Lithium Amitriptyline Desipramine Doxepin	0.8–1.2 mEq/L 120–150 ng/mL 150–300 ng/mL 50–200 ng/mL

for this medication as the trough value has little correlation with the dose or therapeutic effect. The plasma concentration 2 (C2) hours following dosing may be the best measure for this medication (Jirasiritham et al., 2004). Note this C2 approach is also used for the immunosuppressant agent cyclosporine (Marcén et al., 2005). The methods for assessment of the plasma concentration include HPLC, GC/MS (or tandem MS), and automated immunoassays. Both accuracy and cost effectiveness must be considered for large-scale clinical use (Touw et al., 2005). Many of the automated immunoassay units focus on digoxin (ng/mL) and theophylline (mg/mL). Digitalis has difficult onset to effects (1 hour post administration) and a very narrow therapeutic range (0.5–1.5 or 2.0 ng/mL, a long half-life (160 hours) and interacts with many medications, so it must be monitored often and carefully. Any overdose may affect the heart and nervous system severely ( $3\text{Na}^+$ ,  $2\text{K}^+$ -ATPase inhibition), so a digitalis antibody is used to avoid plasma concentrations in excess of 2.0 ng/mL. Automated methods in therapeutic drug monitoring reflect clearly established guidelines for use in a widespread and diverse population. Other medications have controversial aspects of when to measure or whether to measure at all. That is an easier decision to make if the therapeutic activity is more easily determined than previously thought when monitoring was considered. Additionally, the toxicity may be easily observed and does not endanger the patient more than the adverse effects of other medications that are not monitored by plasma concentrations. This is the reason that some doctors in rural areas use the effect of digitalis on heart rate alone as an inexpensive alternative to testing.

## Questions

1. How would you use toxidromes to discern the difference between someone driving under the influence of alcohol (ethanol) versus Vicodin (hydrocodone = opioid + acetaminophen)? How would CO poisoning look different from CN poisoning?
2. What samples should be taken for: (a) a child living in a house manufacturing methamphetamine but not taking any of the drug, (b) monitoring the level of digitalis in a patient, (c) a living person to show that they had used illegal drugs in the recent past, (d) a corpse buried in the ground that might have been poisoned with an organophosphate nerve agent, and (e) chronic exposure of a child to pica (Pb in paint chips)?
3. Why not test for original toxicant, medications, or peptide hormones in urine?
4. In plane crash cases, why not use the urine as this fluid is most likely to contain metabolites of drugs, ethanol, and other prescription medications used before the crash as well as whether the person had diabetes mellitus under control?
5. In therapeutic drug monitoring, when is  $C_{\text{trough}}$  appropriate to use for dosing versus C2 versus establishing a range with maximum and minimum plasma concentrations?

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