

Casarett & Doull's Essentials of Toxicology, 2e >

Chapter 31. Analytic/Forensic Toxicology

Key Points

- Analytic toxicology involves the application of the tools of analytic chemistry to the qualitative and/or quantitative estimation of chemicals that may exert adverse effects on living organisms.
- Forensic toxicology involves the use of toxicology for the purposes of the law; by far the most common application is to identify any chemical that may serve as a causative agent in inflicting death or injury on humans or in causing damage to property.
- The toxicologic investigation of a poison death involves (1) obtaining the case history in as much detail as possible and gathering suitable specimens, (2) conducting suitable toxicologic analyses based on the available specimens, and (3) the interpretation of the analytic findings.
- The toxicologist as an expert witness may provide two objectives: testimony and opinion. Objective testimony usually involves a description of analytic methods and findings. When a toxicologist testifies as to the interpretation of analytic results, that toxicologist is offering an “opinion.”

Analytic Toxicology

Analytic toxicology applies the tools of analytic chemistry to the qualitative and/or quantitative estimation of chemicals that may exert adverse effects on living organisms. Forensic toxicology involves the use of toxicology for the purposes of the law; by far the most common application is to identify any chemical that may serve as a causative agent in inflicting death or injury on humans or in causing damage to property. A systematic approach, a reliance on the practical experience of generations of forensic toxicologists, and use of the sophisticated tools of analytic chemistry provide the data needed to understand better the hazards of toxic substances.

In 1873 in his *Elements de Toxicologie*, Chapuis described a system of classifying toxic agents by their origin or nature into several categories: gases, volatile substances, corrosive agents, metals, anions and nonmetals, nonvolatile organic substances, and miscellaneous. Closely related to this descriptive classification is the method for separating a toxic agent from the matrix in which it is embedded. The agent of interest may exist in a matrix of a simple solution or may be bound to protein and other cellular constituents; before analysis can be done, the agent must be isolated.

Analytic Role in Forensic Toxicology

The duties of a forensic toxicologist in postmortem investigations include the qualitative and quantitative analyses of drugs or poisons in biological specimens collected at autopsy and the interpretation of the analytic findings in regard to the physiologic and behavioral effects of the detected chemicals on the deceased at the time of death.

Establishing the cause of death rests with the medical examiner, coroner, or pathologist, but success in arriving at the correct conclusion often depends on the combined efforts of the pathologist and the toxicologist. The cause of death in cases of poisoning cannot be proved beyond contention without a toxicologic analysis that establishes the presence of the toxicant in the tissues and body fluids of the deceased. Additionally, a toxicologist can furnish valuable evidence concerning the circumstances surrounding a death. Such cases commonly involve demonstrating the presence of intoxicating concentrations of ethanol in victims of automotive or industrial accidents, or determining concentrations of carbon monoxide in fire victims in order to determine whether the deceased died as a result of the fire or was dead before the fire started.

Toxicologic Investigation of a Poison Death

The toxicologic investigation of a poison death may be divided into three steps: (1) obtaining the case history in as much detail as possible and gathering suitable specimens, (2) conducting suitable toxicologic analyses based on the available specimens, and (3) the interpretation of the analytic

findings. Knowledge of drug biotransformation often is essential before analysis is performed because the metabolites may provide the only evidence that a drug or poison has been administered. Specific questions may be answered, such as the route of administration, the dose administered, and whether the concentration of the toxicant present was sufficient to cause death or alter the decedent's actions enough to cause his or her death.

Criminal Poisoning of the Living

Forensic toxicologists have become more and more involved in the analysis of specimens obtained from living victims of criminal poisonings. This increase in testing has arisen from two types of cases: (1) administration of drugs to incapacitate victims of kidnapping, robbery, or sexual assault and (2) poisoning as a form of child abuse.

Common drugs of abuse or other psychoactive drugs that are often involved in cases of alleged sexual assault are listed in Table 31-1. The many potent inductive agents administered prior to general anesthesia are of particular concern. Many of these drugs, such as benzodiazepines and phenothiazines, are available today through illegal and legal sources. When administered surreptitiously, they cause sedation and incapacitate the victim in addition to producing amnesia, without causing severe central nervous system depression. These cases often present a difficult analytic challenge to the toxicologist because the drug has usually been eliminated from the body by the time the victim can bring forth an allegation.

Table 31-1 Distribution of drugs of abuse encountered in urine specimens in 578 cases of alleged sexual assault.

Rank	Drug/Drug Group	Incidence	Percent of Cases*
1	No drugs found	167	29
2	Ethanol	148	26
3	Benzodiazepines	70	12
4	Marijuana	67	12
5	Amphetamines	41	7
6	Gamma-hydroxybutyrate	24	4
7	Opiate (morphine/codeine)	20	4
8	Other drugs	13	3

* Percentages do not add to 100% due to rounding.

Source: Data from ElSohly MA, et al: *Analysis of flunitrazepam metabolites and other substances in alleged cases of sexual assault. Presentation at the 50th Anniversary Meeting of the American Academy of Forensic Sciences, San Francisco, CA, February 13, 1997.*

Poisoning as a form of child abuse involves the deliberate administration of toxic or injurious substances to a child. Common agents used to intentionally poison children have included syrup of ipecac, table salt, laxatives, diuretics, antidepressants, sedative-hypnotics, and narcotics. The poison may be given to an infant to stop its crying or be force-fed to older children as a form of punishment. Sophisticated gas chromatograph/mass spectroscopy testing methods may be required to detect such agents.

Forensic Urine Drug Testing

Concerns about the potentially adverse consequences of substance abuse both for the individual and for society have led to widespread urine analysis for the detection of controlled or illicit drugs. Forensic urine drug testing (FUDT) differs from other areas of forensic toxicology in that urine is the only

specimen analyzed and testing is performed for a limited number of drugs. Initial testing is performed by immunoassays on high-speed, large-throughput analyzers. A confirmation analysis in FUDT-certified laboratories is then performed.

Many individuals who are subject to regulated urine testing have devised techniques to mask their drug use either by physiologic means such as the ingestion of diuretics or by attempting to adulterate the specimen directly with bleach, vinegar, or other products that interfere with the initial immunoassay tests. Thus, specimens are routinely tested for adulteration by checking urinary pH, creatinine, and specific gravity and noting any unusual color or smell. Recently a mini-industry has developed to sell various products that are alleged to “fool drug testers” by interfering with the initial or confirmatory drug test. Thus, FUDT laboratories now routinely test not only for drugs of abuse, but also for a wide variety of chemical adulterants. In most instances, a positive test result for adulteration has as serious a consequence as a positive drug test.

Human Performance Testing

Forensic toxicology activities also include the determination of the presence of ethanol and other drugs and chemicals in blood, breath, or other specimens and the evaluation of their role in modifying human performance and behavior. The most common application of human performance testing is to determine driving under the influence of ethanol (DUI) or drugs (DUID). The threshold blood alcohol concentration (BAC) for diminished driving performance of these complex functions in many individuals is as low as 0.04 g/dL, the equivalent of ingestion of two beers within an hour's time. The statutory definition of DUI in the United States is a BAC of either 0.08 or 0.10 g/dL, depending on the particular state law. These concentrations are consistent with diminished performance of complex driving skills in the vast majority of individuals.

During the past decade, there has been growing concern about the deleterious effects of drugs other than ethanol on driving performance. The highest drug-use accident rates are associated with the use of such illicit or controlled drugs as cocaine, benzodiazepines, marijuana, and phencyclidine. Proving that drug use was important in a driving accident is difficult, and there are legal and scientific problems concerning drug concentrations and driving impairment that need to be resolved.

Courtroom Testimony

The forensic toxicologist often is called upon to testify in legal proceedings. The toxicologist is referred to as an “expert witness.” An expert witness may provide two types of testimony: objective testimony and “opinion.” Objective testimony by a toxicologist usually involves a description of his or her analytic methods and findings. When a toxicologist testifies as to the interpretation of his or her analytic results or those of others, that toxicologist is offering an “opinion.” An expert witness is called to provide informed assistance to the jury. The jury, not the expert witness, determines the guilt or innocence of the defendant.

Analytic Role in Clinical Toxicology

In a clinical setting, analytic toxicology can aid in the diagnosis and treatment of toxic incidents as well as in monitoring the effectiveness of treatment regimens by clearly identifying the nature of the toxic exposure and measuring the amount of the toxic substance that has been absorbed. This information, together with the clinical state of the patient, permits a clinician to relate the signs and symptoms observed to the anticipated effects of the toxic agent.

A cardinal rule in the treatment of poisoning cases is to remove any unabsorbed material, limit the absorption of additional poison, and hasten its elimination. The clinical toxicology laboratory assists by monitoring the amount of the toxic agent remaining in circulation or measuring what is excreted. Commonly encountered intoxicants in emergency toxicology testing and the rapid methodologies to detect their presence in serum and/or urine specimens are presented in [Table 31-2](#).

Table 31–2 Commonly encountered drugs and methods for analysis in emergency toxicology.

Drug/Drug Group	Specimen	Analytic Method
Drugs of abuse (amphetamines, cocaine , opiates, phencyclidine)	Urine	Immunoassays
Ethanol	Serum	GC
Benzodiazepines	Urine/serum	Immunoassay/GC/MS
Acetaminophen , salicylates	Serum	Immunoassay/HPLC
Tricyclic antidepressants	Serum	Immunoassay/HPLC
Ibuprofen	Urine/serum	TLC/HPLC
Dextropropoxyphene	Urine	Immunoassay
Fluoxetine	Urine/serum	TLC/HPLC
Barbiturates (50 percent phenobarbital)	Urine/serum	Immunoassay/GC
Diphenhydramine	Urine	TLC

Note: GC = gas chromatography; GC/MS = gas chromatography/mass spectrometry; HPLC = high-pressure liquid chromatography; TLC = thin-layer chromatography.

Source: Data from Year-End 1998 Emergency Department Data from the Drug Abuse Warning Network, Department of Health and Human Services, December 1999.

Primary examples of the usefulness of emergency toxicology testing are the rapid quantitative determination of **acetaminophen**, salicylate, alcohols, and glycol serum concentrations in instances of suspected overdose. Ethanol is the most common agent encountered in emergency toxicology; serum values are important in the assessment of behavioral and neurologic function. Intoxications from accidental or deliberate ingestion of other alcohols or glycols—such as methanol from windshield deicer or paint thinner, isopropanol from rubbing **alcohol**, and ethylene glycol from antifreeze—are also encountered in emergency departments.

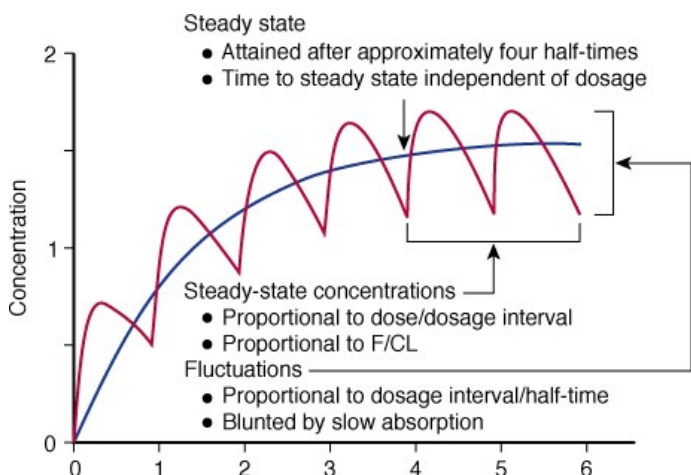
The utilization of the analytic clinical toxicology laboratory has increased enormously in recent years. Typically, the laboratory performs testing for the emergency department and other medical departments, because drugs and toxic agents may be a consideration in diagnosis in many medical problems.

Analytic Role in Therapeutic Monitoring

Historically, the administration of drugs for long-term therapy was based largely on experience. If the drug seemed ineffective, the dose was increased; if toxicity developed, the dose was decreased or the frequency of dosing was altered. The factors responsible for individual variability in responses to drug therapy include the rate and extent of drug absorption, distribution and binding in body tissues and fluids, rate of metabolism and excretion, pathological conditions, and interaction with other drugs. Monitoring of the plasma or serum concentration at regular intervals will detect deviations from the average serum concentration, which indicates that dosage correction is needed.

In a given patient, the administration of the same dose of a drug at regular intervals eventually produces a steady-state condition (**Figure 31–1**). Monitoring of steady-state drug concentrations assures that an effective concentration is present. Appropriate situations for therapeutic drug monitoring are presented in **Table 31–3**.

Figure 31-1



Source: Klaassen CD, Watkins JB: *Casarett & Doull's Essentials of Toxicology, 2nd Edition*: <http://www.accesspharmacy.com>
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Fundamental pharmacokinetic relationships for the repeated administration of drugs. The red line is the pattern of drug accumulation during the repeated administration of a drug at intervals equal to its elimination half-time, when drug absorption is 10 times as rapid as elimination. As the relative rate of absorption increases, the concentration maxima approach 2 and minima approach 1 during steady state. The blue line depicts the pattern during administration of equivalent dosage by continuous intravenous infusion. Curves are based on the one-compartment model. Average concentration (C_{ss}) when steady state is attained during intermittent drug administration is $C_{ss} = F \times \text{dose} / \text{Cl} \times T$, where F is the fractional bioavailability of the dose and T the dosage interval (time). By substitution of infusion rate for $F \times \text{dose} / T$, the formula provides the concentration maintained at steady state during continuous intravenous infusion. (Reproduced with permission from Brunton LL, Lazzo JS, Parker KL: *Goodman & Gilman's the pharmacological basis of therapeutics*, 11th edition. New York: McGraw-Hill, 2006.)

Table 31–3 Appropriate use of therapeutic drug monitoring.

Use	Examples
Optimize efficacy while minimizing toxicity	
Optimal SDC for clinical effect	
Routine, prophylactic peak serum	Aminoglycosides
Poor patient response	Antiarrhythmics, antidepressants
Suspected toxicity	
Resolve complicating factors	
Patient characteristics	Age, smoking, noncompliance
Disease	Renal failure, hepatic disorders
Drug interactions	Induction or inhibition of drug metabolism
Sudden change in physiologic state	Improved cardiac function on lidocaine therapy increases clearance
Dosage regimen design	
Individualize future dosing (single SDC)	
Pharmacokinetic profiling (multiple SDC)	Ideal dosage for aminoglycosides
Follow-up SDC	Single steady-state lidocaine
Verify therapy	Medicolegal lithium

Note: SDC = serum drug concentration.

Because the nature of drugs is varied, many different analytic techniques may be applied. Drugs that are commonly monitored during therapy, usual effective therapeutic serum concentrations, panic values, and typical analytic methodologies applied to serum measurements are presented in [Table 31–4](#). The term *panic value* denotes a serum drug concentration associated with potentially serious toxicity. In clinical laboratories, the panic value alerts the toxicologist to *immediately* notify the treating physician of the test result.

Table 31–4 Drugs commonly indicated for therapeutic monitoring.

Therapeutic Use Drug	Effective Serum Range, mg/L	Panic Value, mg/L	Analytic Methodology
Antiarrhythmic			
Digoxin	0.0005–0.002	0.0024	Immunoassay
Procainamide	4–10	12	Immunoassay

NAPA	5–30	40	Immunoassay
Anticonvulsant			
Carbamazepine	4–12	15	Immunoassay
Gabapentin	2–15	20	GC
Lamotrigine	0.5–8	10	HPLC
Phenobarbital	15–30	50	Immunoassay
Phenytoin	10–20	40	Immunoassay
Tropiramate	2–10	Undetermined	GC
Valproic acid	50–100	200	GC
Antidepressants			
Amitriptyline	0.08–0.250	0.5	HPLC
Desipramine	0.125–0.30	0.4	HPLC
Nortriptyline	0.08–0.250	0.5	HPLC
Antimicrobials			
Tobramycin	0.5–1.5 (trough)	2	Immunoassay
	5–10 (peak)	12	Immunoassay
Vancomycin	5–10 (trough)	90	Immunoassay
	30–40 (peak)		Immunoassay
Immunosuppressant			
Cyclosporine	0.1 (trough, whole blood)		HPLC
Neonatal apnea			
Caffeine	8–20	50	HPLC

Note: GC = gas chromatography; HPLC = high-pressure liquid chromatography.

Analytic Role in Biological Monitoring

Biological monitoring of a worker directly can be a better indicator of exposure than simply monitoring the environment, because the monitoring can show what has actually been absorbed. Often, environmental exposures are to a mixture of chemicals and/or those compounds that are converted to

physiologically important metabolites. Thus, analytic methods must be capable of separating a family of chemical agents and their major metabolites. Additionally, methods must be sufficiently specific and sensitive to measure minute concentrations of the compounds in complex biological matrices.

Besides measuring the chemical or its metabolites in the body fluids, hair, or breath of the worker, other, more indirect methods may be employed. Agents that interact with macromolecules may form adducts that persist for long periods. These adducts can be sampled periodically and potentially can serve as a means of integrating exposure to certain substances over long periods. For example, adducts of ethylene oxide with DNA or hemoglobin have been noted in workers. Another approach that is useful in biological monitoring is to measure changes of normal metabolites induced by xenobiotics. Although monitoring the alteration of the urinary excretion of these metabolites may not indicate exposure to specific substances, this technique can be used in a generic fashion to **flag** a potentially harmful exposure. The early recognition of a toxicologic problem may permit the protection of a worker before irreversible effects occur.

Conclusion

Though analytic techniques expand in complexity and improve in reliability, forensic toxicologists continue focusing on conducting unequivocal identification of toxic substances in such a manner that the results can withstand a legal challenge. Substance abuse, designer drugs, increased potency of therapeutic agents, and concern about pollution and the safety and health of workers will continue to present challenges to the analyst's skills.

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