CLINICAL & FORENSIC

Toxicology News

September 2013 *An AACC/CAP Educational Newsletter for Toxicology Laboratories*

Pediatric Toxicology

Labs Play Important Role in Poisonings

By Jennifer A. Lowry, MD

Poisonings are a leading and growing cause of injury and mortality among all age groups. They occur in a wide range of circumstances, including overdoses of illicit drugs, environmental exposures, suicides and suicide attempts, homicides, unintentional medication misuse, and accidental ingestion of household products.

The Centers for Disease Control and Prevention reports that the death rate from drug poisoning quadrupled between 1999 and 2008 (1). Drug poisoning was the leading cause of injury deaths in 2008, exceeding even motor vehicle crashes. Unintentional poisoning is the tenth leading cause of nonfatal injuries seen in emergency departments, but among children ages one to four, it is the second leading cause.

Our approach to pediatric toxicology has changed over the years. The focus on preventing unintentional ingestions in young children through measures such as child-resistant closures on pill bottles combined with the emergence of poison control centers has greatly reduced poisoning morbidity and mortality in young children. With that success, pediatric toxicology has evolved to include issues such as law enforcement and environmental and public health.

Poison Surveillance

According to data submitted to the American Association of Poison Control Centers, in 2011, 57 poison control centers (PCCs) cared for 2,333,004 human exposures (2). Some 1,449,186 (62%) of these concerned children 19 and under. These figures undoubtedly greatly underestimate the number of poisonings nationwide because poison control centers rely on voluntary reporting.

The vast majority of calls to poison centers involved exposures in residences. About 18% of calls to PCCs were from healthcare professionals after patients presented at their facilities. Ingestions accounted for 83% of exposures, compared with 7% for dermal and 6.1% for inhalation routes.

Management largely occurred at the site of exposure (69%), most often at the patient's residence. Approximately 25% of patients were seen in an emergency department, but many of them could have avoided emergency room care if they had called a PCC first. Most patients seen in an emergency department were treated and released, and about one-quarter of them were admitted for hospitalization.

Children tend to fall victim to readily available substances. The most common categories are cosmetics and personal care products (14%), analgesics (10%), household cleaning substances (9%), foreign bodies (7%), and topical preparations (7%). With the exception of analgesics, these products do not generally result in significant toxicity. Although less likely to be ingested in the younger age group, prescription drugs can have significant effects. The level of toxicity is associated with the child's intent, and the reasons for exposures change with age.

Young Children

Children younger than three were involved in 36% of exposures, and those younger than six accounted for about half of all human exposures (49%) called to PCCs. Of the pediatric exposures, about 80% occur in children under the age of 6 years, with the majority unintentional.

Ingestions in young children often result from the development of exploration behaviors and hand-to-mouth activity resulting in the ingestion of pills,

Continued on page 2

Inside...

Pediatric Toxicology

Continued from page 1

toys, paint chips, and other substances. Pediatricians and other healthcare professionals are encouraged to discuss these risks at the one-year well-child examination as guidance for poisoning prevention.

Although most ingestions result in minor symptoms, young children are at higher risk for adverse effects. Small quantities of some drugs cause little harm, but some agents may cause morbidity and mortality (3). Dangerous substances include calcium channel blockers, centrally acting alpha agonists, methylsalicylate, sulfonylureas, tricyclic antidepressants, chloroquine, diphenoxylate-atropine, toxic alcohols, and methadone. When a child presents with unexplained symptoms, healthcare providers must consider poisoning and understand its signs and symptoms to provide appropriate and timely care.

The prescription drug abuse epidemic has led to more unintentional exposures of young children. For example, from 2002 to 2008, the number of buprenorphine exposures in children younger than six reported to PCCs increased from two to 907 cases, while methadone exposures doubled from 155 to 332 cases (4). The number of buprenorphine exposures now greatly exceeds methadone exposures in these children, reflecting the growth in the use of this drug.

Growing evidence shows that childhood environmental exposures in the home, at school, and during recreation cause adverse health effects that can continue into adolescence and adulthood (5). These exposures include environmental tobacco smoke, allergens, bioaerosols, chemicals in the indoor and outdoor environments, carbon monoxide, and particulate matter. They are associated with disorders such as asthma, obesity, and adult cardiac disease. In addition, environmental toxins such as lead cause neurodevelopmental effects. Although the number of children with elevated lead levels is declining, the CDC recently decreased the reference blood level for action to 5 µg/dL because of new evidence associating even low lead levels with adverse effects on children's attention and cognition (6).

Adolescents

As children age, the reason for poisoning shifts from unintentional to intentional exposure.

Children over 12 are more likely to intentionally ingest drugs for abuse and suicide. One of the best long-term studies of adolescent substance use is the Monitoring the Future Survey, conducted annually by the University of Michigan's Institute for Social Research since 1975 and supported by the National Institute on Drug Abuse (7). In 2012, the program surveyed some 45,400 8th-, 10th-, and 12th-grade students nationwide.

The most recent data suggests decreased drug use among high-school teenagers, but rates of marijuana and nonmedical prescription drug use remain high. For example, 7.5% of high-school seniors use unprescribed hydrocodone. The nonmedical use of prescription stimulants such as Adderall by seniors increased from 5.4% in 2009 to 7.6% in 2012. In addition, 68% of 12th graders admit to obtaining prescription pain relievers for free from friends and family.

Although home exposures in adolescents resemble those in younger children, adolescents tend to spend less time at home and more time in school, work, and play activities, where they can encounter poisoning agents. They spend significant time at school, and indoor air quality is correlated with school performance (5).

More than 30% of high-school-age children work during the school year and summer. This work commonly includes janitorial, grocery store, restaurant, landscaping, and construction jobs where these untrained workers can be exposed to chemicals. Injuries and poisonings are not uncommon and may be higher than in adults with more experience. Adolescents are less likely to wear personal protective gear, which increases their risk for exposure.

Pediatric Fatalities

According to the CDC, the age-adjusted drug poisoning death rate nearly doubled from 2000 to 2010 (1). The CDC National Vital Statistics Report says that in 2010, 832 unintentional and 121 suicide poisoning deaths occurred in children between 1 and 19 years of age (Figures 1 and 2) (8). Notably, in children 12–19 years of age, unintentional poisoning deaths were second only to motor vehicle accidents as a cause of "unintentional injury" deaths (Figure 3).

The CDC figures contrast with those from PCCs, which reported only 70 deaths in the pediatric age group in 2011. As previously mentioned, pharmaceutical products were involved more often than non-pharmaceutical products. Analgesics were the most common drugs associated with pediatric deaths (21%), followed by stimulants and street drugs (12%), then cold and cough preparations (10%). Of the pediatric deaths, 63% occurred in children under six. Notably, several cases were due to the lack of child-resistant packaging on medications for older adults. Of the deaths in children over the age of six, 46% were suicides and 15% from substance abuse.

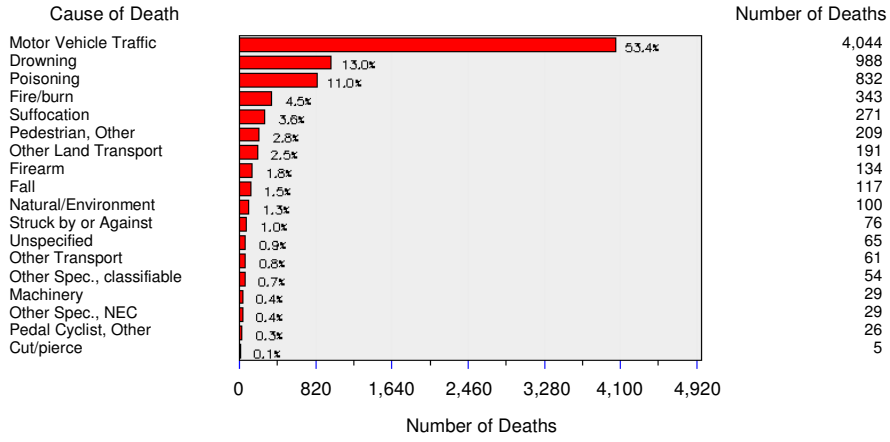


Figure 1. Unintentional Injuries in the U.S., 2010, Ages 1–19, Total Deaths = 7,574
NEC = Not Elsewhere Classifiable
Produced by National Center for Injury Prevention and Control, Centers for Disease Control and Prevention; Data Source: National Center for Health Statistics, National Vital Statistics System

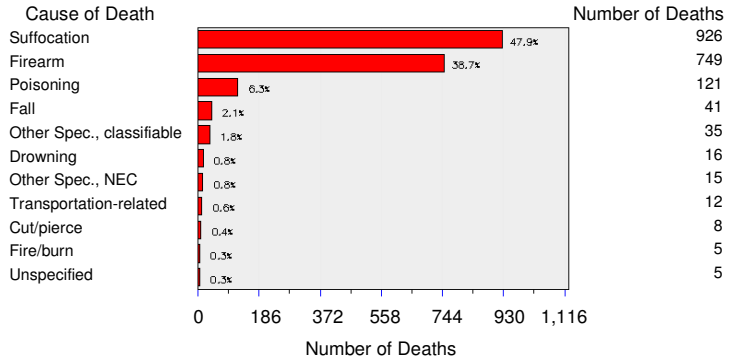


Figure 2. Suicides in the U.S., 2010, Ages 1–19, Total Deaths = 1,933
NEC = Not Elsewhere Classifiable
Produced by National Center for Injury Prevention and Control, Centers for Disease Control and Prevention; Data Source: National Center for Health Statistics, National Vital Statistics System

The ratios in the poison center data are the opposite of those reported by the CDC (9).

The danger of pediatric deaths from the use of over-the-counter cough and cold preparations led a Food and Drug Administration advisory panel to recommend prohibiting their use in children younger than six. In response to this recommendation, a panel of medical toxicologists and clinical pharmacologists assessed 189 cases and concluded that fatalities from these preparations were uncommon and most were associated with overdoses rather than therapeutic doses (10). Most fatalities occurred in children under two and were associated with malicious intent or an attempt to induce sedation. The authors agreed with the recommendation to limit the use of these medications.

Lab Assessment of the Poisoned Child

Laboratory tests can contribute to the diagnosis and management of the poisoned child. When the poisoning agent is unknown, laboratory results can provide key evidence for the astute practitioner. Patients with altered mental status or who have ingested an agent that could result in hypoglycemia should have their blood glucose concentration measured. Hypoglycemia is one of the most easily detected and treated effects of poisons, so this is one of the most commonly ordered tests.

Other chemistry tests that can be help guide care and identify the poisoning agent include serum electrolytes and a calculated anion gap. Poisons that result in a high anion gap include salicylates, ethanol, ethylene glycol, methanol, isoniazid, and iron (Table 1). Females of child-bearing age also should have a pregnancy test.

Laboratories offer a variety of quantitative drug and toxin assays for which serum concentration correlates with clinical effect and toxicity (Table 2). Any pediatric patients who present with an overdose should be tested for acetaminophen and salicylate serum concentrations because these drugs are common offenders, are often co-ingested, and may not exhibit early diagnostic clues.

Screening for lead poisoning is routine in children at the 1- and 2-year well-child exams. In fact, the CDC recommends annual blood lead concentration measurements for all children under six.

Most labs have the ability to assess serum iron concentration to check for anemia or iron overdose. Fewer labs offer the ability to test for other heavy metals such as mercury, so these samples may be sent to a reference laboratory.

The diagnosis of a poisoned child should largely be based on the presenting history, physical exam, and basic laboratory results.

Standard Drug Panel

The standard toxicological screening panel for drugs commonly abused by adults—such as marijuana, opiates, amphetamines, benzodiazepines, and cocaine—rarely offers new, unsuspected

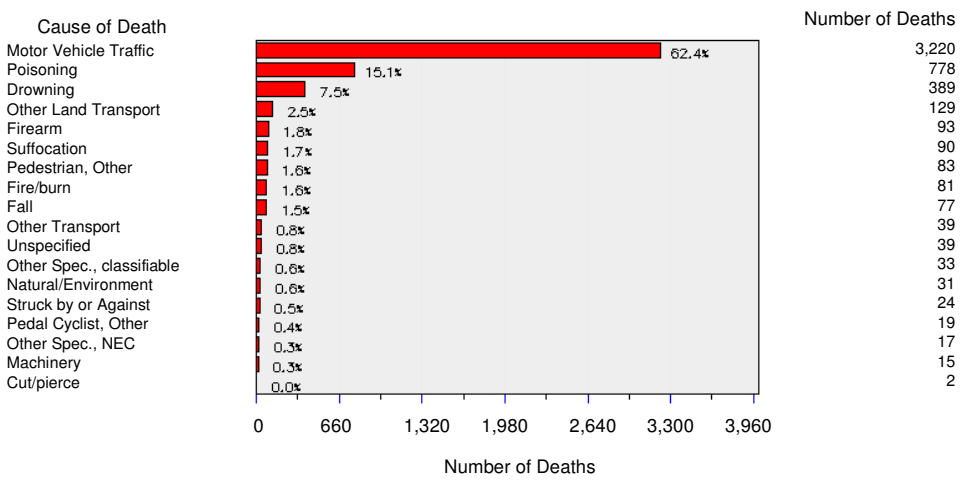


Figure 3. Unintentional Injuries in the U.S., 2010, Ages 12–19, Total Deaths = 5,159
NEC = Not Elsewhere Classifiable
Produced by National Center for Injury Prevention and Control, Centers for Disease Control and Prevention; Data Source: National Center for Health Statistics, National Vital Statistics System

Table 1. Agents that Cause an Elevated Anion Gap

Methanol, Metformin
Uremia
Diabetic ketoacidosis
Paraldehyde, Phenformin
Isoniazid, Iron, Ibuprofen
Lactic acid
Ethanol, Ethylene glycol
Salicylates

Many healthcare professionals use the mnemonic MUDPILES to remember this list.

Source: Reference 11

Table 2. Useful Quantitative Drug and Toxin Assays

Acetaminophen
Anticonvulsants (carbamazepine, phenytoin, valproic acid)
Barbiturates
Carbon monoxide
Digoxin
Ethanol
Ethylene glycol
Iron
Lead and other heavy metals
Lithium
Methanol
Methemoglobin
Salicylates

Source: Reference 11

information about the pediatric poisoning patient, in part because children are not likely to have been exposed to these drugs.

In addition, the tests can have problems with false-positive and false-negative results. For some drug classes, the reference drug's structure differs from that of commonly used drugs, resulting in a negative drug screen. Therefore, opiate screens that key on morphine may not detect oxycodone. Conversely, some pharmaceuticals have structures similar to those of drugs, so dextromethrophan can trigger a false-positive phencyclidine (PCP) result.

Most of the pharmaceuticals and chemicals a child is likely to be exposed to do not cause a positive urine drug screen, and a negative result could lead to the erroneous belief that no poisoning occurred, suggesting that these tests may not be useful in pediatrics.

Management of the Poisoned Child

Management of poisonings should begin with the ABCs of medicine—airway, breathing, and circulation. Decontamination with activated charcoal should be considered in the child who presents with a potentially harmful dose within an hour of the ingestion, has not ingested a hydrocarbon or seizure-causing medication, and is awake, alert, and maintaining the airway. Activated charcoal is not without complications, including very serious ones, such as chemical pneumonitis. The use of an emetic, such as syrup of ipecac, is no longer recommended.

Treatment of poisoning is largely supportive and based on the alleviation of symptoms. The key is recognizing that the patient has been poisoned and, if the agent is unknown, determining the etiology by the history, physical exam, and basic laboratory analysis. Consultation with a PCC or a medical toxicologist is advised. Antidotes are available for few poisons and should not be used indiscriminately because they can complicate the clinical situation.

Conclusion

Appropriate laboratory tests are critical to diagnosing and managing poisoned children, whose small size makes them especially vulnerable physically to the effects of poisons. Laboratory studies should be performed based on clinical presentation and history. Support from poison centers and medical toxicologists is strongly advised.

References

1. Warner M, Chen LH, Makuc DM, et al. Drug poisoning deaths in the United States, 1980–2008. NCHS Data Brief 2011;81:1–8.
2. Bronstein AC, Spyker DA, Cantilena LR Jr, et al. 2011 Annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS). Clin Toxicol 2012;50:911–1164.
3. Henry K, Harris CR. Deadly ingestions. Pediatr Clin North Amer 2006;53:293–315.
4. Boyer EW, McCance-Katz EF, Marcus S. Methadone and buprenorphine toxicity in children. Am J Addict 2009;9:89–95.
5. Anderson ME, Bogdan GM. Environments, indoor air quality and children. Pediatr Clin North Amer 2007;54:295–307.
6. Centers for Disease Control and Prevention. Blood lead levels in children aged 1–5 years—United States, 1999–2010. MMWR Morb Mortal Wkly Rep 2013;62:245–8.
7. Institute for Social Research. University of Michigan. www.monitoringthefuture.com (Accessed July 2013).
8. Centers for Disease Control and Prevention. Vital signs: unintentional injury deaths among persons aged 0–19 years of age in the United States, 2000–2009. MMWR Morb Mortal Wkly Rep 2012;61:1–7.
9. Fine JS, Callelo DP, Marcus SM, et al. 2011 Pediatric fatality review of the National Poison Data System. Clin Toxicol 2012;50:872–4.
10. Dart RC, Paul IM, Bond GR, et al. Pediatric fatalities associated with over the counter (nonprescription) cough and cold medications. Ann Emerg Med. 2009;53:411–7.
11. Nelson LS, Lewin NA, Howland MA, et al., eds. Goldfrank's toxicologic emergencies. 9th Ed. New York: McGraw-Hill Professional 2011.

Jennifer A. Lowry, MD, is chief of the section of clinical toxicology at Children's Mercy Hospital and an associate professor in the department of pediatrics at the University of Missouri–Kansas City School of Medicine. Email: jlowry@cmh.edu

Disclosure: The author has nothing to disclose.

Watch for the next issue of *CFTN* in December

Mushrooms: Magic or Deadly?

Two Types Cause Most Poisonings

By Barbarajean Magnani, PhD, MD

Although many toxic species of mushrooms grow throughout the world, two types cause the most poisonings.

Users call *Psilocybe* species "magic mushrooms," and ingest them recreationally because the psilocybin and psilocin they contain are considered hallucinogenic. In contrast, people generally eat *Amanita phalloides*, or death cap mushrooms, by accident. They contain amatoxins and phallotoxins, powerful poisons that can cause death.

Given this knowledge, the most important consideration in mushroom poisoning is patient history. The time the individual consumed the mushrooms, the number eaten, the time of onset of symptoms, and the mushroom itself (or a description) are all important in determining treatment, particularly in the case of *Amanita* poisoning.

Psilocybe sp. Characteristics

Psilocybe mushrooms have light-colored caps, elongated stalks, and dark gills and spores. Some species produce a bluish green color when the flesh is crushed, but this characteristic is not consistent among the different species.

Psilocybin is a serotonin-like compound with properties similar to those of LSD. Consequently, users ingest these mushrooms seeking euphoria, including optical, auditory, and tactile illusions. The species' major metabolite, psilocin, is created through dephosphorylation, and both psilocybin and psilocin are present together. Psilocybin content varies among species from roughly 0.1mg/g to 10 mg/g of dried flesh. Users may ingest from 20 to 100 mushrooms at a time, enough to cause psilocybin overdose. The hallucinogenic effects begin about 15 minutes after ingestion, and the peak lasts about an hour. Although most users' symptoms resolve without medical intervention, those who experience cardiac abnormalities or severe central nervous system symptoms, such as seizures, may seek medical attention.

Amanita phalloides Characteristics

A ubiquitous species that usually grows in summer and autumn months, *Amanita* causes the majority of mushroom poisoning fatalities. Three or four of them weigh about 50 grams, and just 20 to 50 grams of their fresh flesh can produce critical liver and kidney damage.

Patients with *Amanita* toxicity usually present within 6–12 hours of ingestion. The chief symptoms include abdominal pain, nausea, vomiting, and diar-

rhea as frequent as six times per hour. If toxicity proceeds unimpeded, patients progress to acute hepatorenal failure, encephalopathy, coma, and ultimately death.

The amatoxins in *A. phalloides* are thermostable, so cooking, drying, and freezing do not alter their toxicity. The fatal dose of α-amanitin is 0.1–0.3 mg/kg.

Supportive care such as fluid treatment helps, but specific therapy is essential. Gastrointestinal decontamination with whole-bowel irrigation and activated charcoal and specific antidotes can reduce the death rate from the 50–90% experienced by patients who receive only supportive care to below 10%. Antidotes to α-amanitin that can limit toxin absorption include silibinin (extract of milk thistle) and intravenous benzyl penicillin. When treatment does not succeed, liver transplantation may be needed.

A Simple Test Provides a Clue

The Meixner test or newspaper test of Wieland provides a simple but nonspecific test for amanita toxins. It involves blotting a sample of the mushroom on a piece of paper with a high lignin content (such as newspaper), adding concentrated hydrochloric acid, then watching for the development of a blue color. The blue color results from the substituted indole residue in the amanita toxins reacting with the lignin in the paper. However, the test is not specific because other ring-containing compounds, including the psilocin found in *Psilocybe, Panaeolus,* and *Conocybe* mushrooms, also produce the blue color.

Suggested Reading

1. Beuhler M, Graeme K. Overview of mushroom poisoning. In: Brent J, Wallace K, Burkhart K, Phillips S, Donovan J, eds. Critical Care Toxicology. Elsevier Mosby 2005:1263–75.
2. Beuhler M, Lee DC, Gerkin R. The Meixner test in the detection of alpha-amanitin and false-positive reactions caused by psilocin and 5 substituted tryptamines. Ann Emerg Med 2004;44:114–20.
3. Habal R, Martinez JA. Toxicity, mushroom. eMedicine. www.eglobalmed.com/opt/MedicalStudentdotcom/www.emedicine.com/med/topic1527.htm (Accessed July 2013).

Barbarajean Magnani, PhD, MD, is pathologist-in-chief at Tufts Medical Center and a professor at Tufts University School of Medicine in Boston. She is a member of the Clinical & Forensic Toxicology News *editorial advisory board. Email: bjmagnani@tuftsmedicalcenter.org*

Disclosure: The author has nothing to disclose.

Emerging Alcohol Biomarkers

PEth Could Provide New Capabilities

By David J. Kuntz, PhD, DABFT

Some 16 million Americans abuse alcohol, and the societal costs associated with this abuse exceed $185 billion annually.

In the 2011 National Survey on Drug Use and Health, 52% of Americans reported drinking alcohol. About 23% participated in binge drinking (five or more drinks at a time on at least 1 day in the past 30 days) and 6% reported heavy drinking (five or more drinks on five or more days in the past 30 days). About 75% of these binge and heavy alcohol users were employed (1).

Because of the high costs and pervasiveness of alcohol abuse, researchers have developed a series of biomarkers to provide physiological indicators of alcohol ingestion. Alcohol biomarkers can identify liver damage associated with problematic drinking, monitor patients in alcoholism treatment and prevention programs, differentiate social drinking from problem drinking, and identify recent drinking in pregnancy. The newer alcohol biomarkers also correlate to alcohol use patterns for the past 30 days and are far more specific than traditional automated chemistry tests for alcohol abuse.

Indirect Biomarkers

The four indirect blood or serum biochemical markers associated with alcohol abuse, gamma glutamyl transferase (GGT), alanine amino transferase (ALT), mean corpuscular volume (MCV), and carbohydrate-deficient transferrin (CDT), all lack sensitivity or specificity. Liver cell damage associated with alcohol abuse causes elevations in GGT and ALT levels, but a variety of other conditions can cause these elevations, including pancreatitis, myocardial infarction, hepatitis B, hepatitis C, and some prescription drugs. Increases in MCV, or the size of red blood cells, are associated with alcoholism, liver disease, and deficiencies of vitamin B12 and folic acid. Heavy alcohol consumption causes transferrin to lose a carbohydrate residue on some of its terminal chains, which can be picked up by the CDT test. Measured in serum, CDT is sensitive to episodic heavy alcohol use (> 60 grams/day) for 2 weeks, but transferrin isoforms can interfere with results (2–4).

Direct Biomarkers

Laboratories can measure direct biomarkers using gas chromatography-flame ionization detection (GC-FID) and liquid chromatography-tandem mass

spectrometry (LC/MS/MS) rather than by automated chemistry tests. GC-FID detects alcohol ingestion directly by measuring blood alcohol concentration. More advanced techniques, such as LC/MS/MS, detect the newer direct biomarkers.

Fatty Acid Ethyl Esters

One of the newest of these under investigation is fatty acid ethyl esters (FAEEs), which are formed during non-oxidative metabolism of ethanol by the conjugation of ethanol to endogenous free fatty acids and fatty-acid acyl-CoA. They can be detected in hair, meconium, skin surface lipids, and blood. Several studies have attempted to correlate the FAEE concentrations with the amount of alcohol use, but the test cannot detect FAEEs until 1–2 weeks after high alcohol consumption (5,6).

Ethyl Glucuronide and Ethyl Sulfate

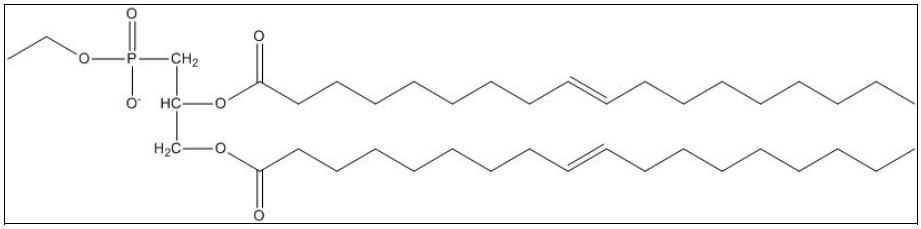
Laboratories measure two alcohol metabolites,ethyl glucuronide and ethyl sulfate, in urine, usually via LC/MS/MS, but other specimens, including hair, can also be used. Both metabolites appear in urine soon after alcohol use and can reach several thousand nanograms per milliliter of urine within 2–4 hours. Tests can detect them for 1 or 2 days following minimal alcohol consumption.

Because alcohol is found in many products, from over-the-counter preparations (such as mouth wash, cough syrups, and alcohol-gel hand sanitizers), liquid prescription drugs, aerosols (such as hair spray and inhalers), food (such as alcohol-based flavorings), and even fermented canned fruits, the source of a low-positive ethyl glucuronide/ethyl sulfate result can be difficult to determine. Recovery programs commonly use this test to monitor abstinence in individuals with a history of alcohol abuse who are in rehabilitation to maintain a professional license (doctors, nurses, pharmacists, and attorneys), for an automobile license suspension, or for underaged drinking (7).

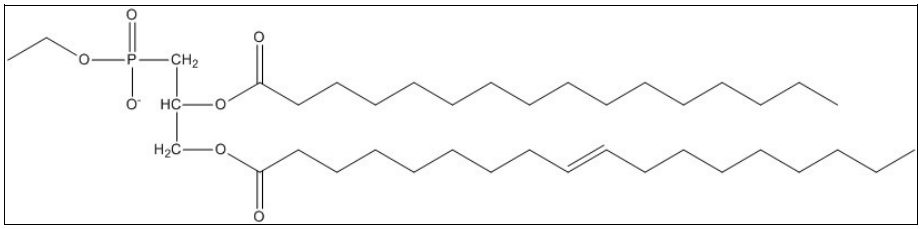
The Promise of Phosphatidylethanol

Phosphatidylethanol (PEth) is an ethanol-derived phospholipid formed primarily in erythrocytes. It is synthesized from phosphatidylcholine in cell membranes by a transphosphatidylation reaction catalyzed by phospholipase D. Phospholipase D normally hydrolyzes phosphatidylcholine into phosphatidic acid and choline. However, because it has a 1000-fold greater affinity for ethanol than water, the presence of ethanol leads to the formation of PEth rather than the normal phosphatidic acid. PEth's half-life ranges from 4.5 to 12 days in social drinkers in blood, with a mean half-life of about 4 days in alcoholics (8).

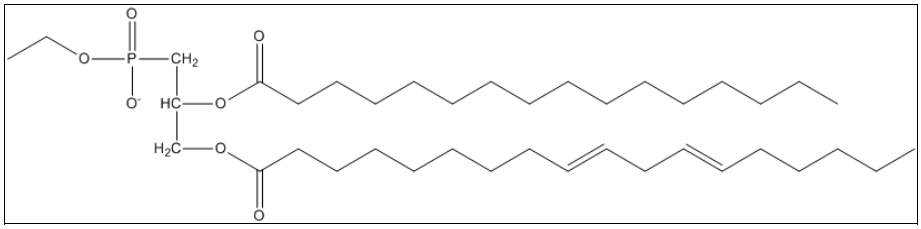
PEth is not a single molecular species but rather a group of phospholipids with a common nonpolar phosphoethanol head group attached with two fatty acid moieties. These moieties have typical chain lengths of 16, 18, or 20 carbons with a number of combinations of double bonds (Figure 1). The major fatty acid combinations monitored are 18:1/18:1, 16:0/18:1, and 16:0/18:2, with the nomenclature indicating the number of carbons:number of double bonds. Analysts extract the fatty acids from erythro-



PEth 18.1/18.1



PEth 16.0/18.1



PEth 16.0/18.2

Figure 1. Phosphatidylethanol (PEth) Structures

Source: Reference 11

cyte membranes and typically employ LC-MS/MS using negative mode electrospray. As an example, transitions monitored for the 16:0/18.1 are *m/z* 701.5 to 281.2 (9–11).

PEth is a promising marker because there appear to be no false-positive results and it provides a clear indication of alcohol use. One study compared the sensitivity of CDT, GGT, MCV, and PEth tests in outpatients and inpatients. When test subjects consumed less than 40 grams of alcohol/day, CDT and GGT had sensitivities of approximately 40%. In the 40–60 grams/day range, their sensitivities increased to 60%. Only when intake surpassed 200 grams/day did the sensitivities of CDT and GGT approach 90%. When CDT, GGT, or MCV were compared individually, CDT was the most sensitive. PEth had a sensitivity of 100% in all patients (12).

In a controlled drinking study, volunteers consumed enough to reach a 1 g/kg blood alcohol level on each of 5 consecutive days followed by 16 days of abstinence (8). PEth was detected within 1 hour after the start of drinking and reached maximum concentrations of 74–237 ng/mL between days 3 and 6. In alcoholics, PEth levels reach 4000 ng/mL. Further studies could make the back-extrapolation of alcohol consumption more accurate with PEth than other biomarkers.

Accurate PEth measurement requires careful storage of the blood sample. Storage for 72 hours at 4°C or at -80°C does not affect measurements, but slight elevations occur at room temperature and at -20°C in blood spiked with ethanol. Dried blood spots provide the most accurate results in specimens that are not analyzed immediately. A comparison of dried blood spots and whole blood using LC/MS/MS found excellent correlations in 100 µL of blood (13,14).

The Promise of New Biomarkers

The laboratory can play a significant role in detecting and monitoring alcohol abuse. Direct markers, such as ethyl glucuronide/ethyl sulfate, offer short detection windows but extreme sensitivity. New biomarkers, such as FAEEs and PEth, can lengthen the window of detection to 30 days.

References

1. Substance Abuse and Mental Health Services Administration. Results from the 2011 National Survey on Drug Use and Health: summary of national findings, NSDUH Series H-44, HHS Publication No. (SMA) 12-4713. Rockville, Md: 2012.
2. Substance Abuse and Mental Health Services Administration Advisory. Role of biomarkers in the treatment of alcohol use disorders, 2012 revision. HHS Publication No. (SMA) 12-4686. 2012;11:1–8.
3. Arndt T. Carbohydrate-deficient transferrin as a marker of chronic alcohol abuse: a critical review of preanalysis, analysis, and interpretation. Clin Chem 2001;47:13–27.
4. Helander A, Eriksson G, Stibler H, et al. Interference of transferrin isoform types with carbohydrate-deficient transferrin quantification in the identification of alcohol abuse. Clin Chem 2001;47:1225–33.
5. Laposata M. Fatty acid ethyl esters: short-term and long-term serum markers of ethanol intake. Clin Chem 1997;43:1527–34.
6. Gonzalez-Illan F, Ojeda-Torres G, Diaz-Vazquez, et al. Detection of fatty acid ethyl esters in skin surface lipids as biomarkers of ethanol consumption in alcoholics, social drinkers, light drinkers, and teetotalers using a methodology based on microwave-assisted extraction followed by solid-phase microextraction and gas chromatography-mass spectrometry. J Anal Toxicol 2011;35:23–7.
7. Kuntz D. Use and application increase for new alcohol markers. Clin & Foren Toxicol News; June 2009:2–4.
8. Gnann H, Weinmann W, Thierauf A. Formation of phosphatidylethanol and its subsequent elimination during an extensive drinking experiment over 5 days. Alcohol Clin Exp Res 2012;36:1507–11.
9. Zheng Y, Beck O, Helander A. Method development for routine liquid chromatography-mass spectrometry measurement of the alcohol biomarker phosphatidylethanol (PEth) in blood. Clin Chim Acta 2011;412:1428–35.
10. Gnann H, Weinmann W, Engelmann C, et al. Selective detection of phosphatidylethanol homologues in blood as biomarkers for alcohol consumption by LC-ESI-MS/MS. J Mass Spectrom 2009;44:1293–9.
11. Helander A, Zheng Y. Molecular species of the alcohol biomarker phosphatidylethanol in human blood measured by LC-MS. Clin Chem 2009;55:1395–1405.
12. Aradottier S, Asanovska G, Gjerss S, et al. Phosphatidylethanol (PEth) concentrations in blood are correlated to reported alcohol intake in alcohol-dependent patients. Alcohol Alcoholism 2006;41:431–7.
13. Faller A, Richter B, Kluge M, et al. LC-MS/MS analysis of phosphatidylethanol in dried blood

spots versus conventional blood specimens. Anal Bioanal Chem 2011;401:1163–6.
14. Aradottir S, Seidl S, Wurst F, et al. Phosphatideylethanol in human organs and blood: a study on autopsy material and influences by storage conditions. Alcohol Clin Exp Res 2004;28:1718–23.

David J. Kuntz, PhD, DABFT, is executive director of analytical toxicology at Clinical Reference Laboratory in Lenexa, Kansas, and a member of the Clinical & Forensic Toxicology News *editorial advisory board. Email: kuntzd@crlcorp.com*

Disclosure: The author has nothing to disclose.

CFTN Readers Eligible to Receive ACCENT Credit

Readers of *Clinical & Forensic Toxicology News* are eligible to receive 4.0 ACCENT® credit hours per year (one credit per quarterly issue) of continuing education. ACCENT credit allows you to document your continuing education to meet requirements for licensure or certification.

ACCENT credit is recognized by a wide variety of organizations, including:

- American Association of Bioanalysts
- American Board of Clinical Chemistry
- American Society of Microbiology
- American Society for Clinical Laboratory Science
- American Society for Clinical Pathology
- American Medical Technologists
- Association of Clinical Scientists
- International Federation of Clinical Chemistry
- National Registry in Clinical Chemistry

Learning Objectives

After reading *Clinical & Forensic Toxicology News*, the reader will be able to:

- Describe emerging and changing trends in drug abuse, including new designer drugs, usage patterns, and contaminants/adulterants.
- Identify potential analytes (drugs, metabolites, biomarkers) of clinical and/or forensic significance.
- Evaluate methodologies for their utility and limitations relative to the needs of toxicology labs.
- Explain the analytical and regulatory issues unique to specific applications, including postmortem toxicology, workplace drug testing, and drug screening.

How to Get Credit

After reading this issue's articles, simply **access the online evaluation form and print your continuing education certificate:** http://direct.aacc.org/customerservice/login.aspx?returnlink=http://apps.aacc.org/applications/apps2/CE/intro.aspx?actNum=517686

Clinical & Forensic Toxicology News provides practical and timely information on the clinical, forensic, technical, and regulatory issues faced by toxicology laboratories. Each issue includes article authored by experts

Clinical & Forensic Toxicology News is an educational service of the Forensic Urine Drug Testing (FUDT) Accreditation Program. Cosponsored by the American Association for Clinical Chemistry and the College of American Pathologists, the program includes three components: FUDT accreditation, the FUDT proficiency testing survey, and this newsletter. The accreditation program is the responsibility of CAP. The surveys are sponsored jointly by AACC and CAP. The digital newsletter is published quarterly by AACC, 1850 K St., N.W., Suite 625, Washington, DC 20006, (800) 892-1400 or (202) 857-0717. Editor: Nancy Sasavage, PhD, Email: nsasavage@aacc.org.

Clinical & Forensic Toxicology News does not accept advertising and is supported solely by its readers. The annual subscription price is $65, $45 for AACC members.

Opinions expressed are those of the authors and do not represent the position of AACC or CAP.