

Toxicology News

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Urine Alcohol Testing is a Valuable, Underused Tool

By Leo Kadehjian

Why is urine alcohol testing not more widely used given the large body of supportive literature? In the 1940s, breath alcohol testing became important in drunk driving offenses because of its ease of specimen collection. The correlations of breath and blood concentrations to impairment were established and codified into state laws. Yet the first appellate court decision upholding the admissibility of alcohol test results in a drunk driving offense involved a urine alcohol result (1).

Urine has long been recognized as a suitable specimen for the detection and measurement of ethanol, with the first scientific publications as early as 1877, and the famous alcohol researcher Widmark publishing on urine alcohol as early as 1915. Today, urine specimens are routinely collected in a variety of drug-testing programs and more consideration should be given to the utility of using these specimens for alcohol testing.

There have been two primary concerns about the use of urine for alcohol testing:

- the possibility of ethanol forming in urine specimens from the fermentation of glucose in microbial-infected specimens, and
- the concern that the urine:blood ratio is too variable for forensic applications.

A review of the scientific and clinical literature demonstrates that these concerns are exaggerated.

Analytical issues

Ethanol levels in urine can be accurately measured by a variety of analytical techniques, most commonly gas chromatography or enzymatic methods using alcohol dehydrogenase. The enzymatic methods, introduced 50 years ago, have also proven suitable for non-instrumental on-site testing. The specificity of alcohol dehydrogenase for ethanol is excel-

lent, with no clinically significant cross-reactivity to other volatiles. There is no question that these methods meet evidentiary standards for accuracy and precision. Furthermore, ethanol levels in properly stored urine specimens have also been shown to be stable with no significant changes even over extended periods (2).

Urine ethanol from fermentation

One widely expressed concern regarding the use of urine specimens has been the possibility that ethanol could form as a result of fermentation in microbial-infected specimens that also contain glucose. This possibility was mentioned as early as 1926. Urine samples containing glucose, such as from diabetics, and infected by certain microorganisms, such as *Candida albicans*, have been shown to produce ethanol when stored for several days at room temperature. However, no study has demonstrated any significant ethanol formation in glucose-containing and infected specimens before a storage time of at least one day at room temperature. Thus, proper specimen handling can eliminate this concern.

If specimens will not be tested within one day, they should be refrigerated. Alternatively, the addition of 1% NaF has been demonstrated to minimize the possibility of fermentation (3).

One laboratory noted in its newsletter that it tests all ethanol-positive urine specimens for glucose. Unfortunately, measuring urine glucose levels would not refute any challenge. If glucose is present, it does not prove that any alcohol came from fermentation. If no glucose is found, one can simply argue that the alcohol came from fermentation of glucose that as a result has been completely consumed.

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Urine Alcohol

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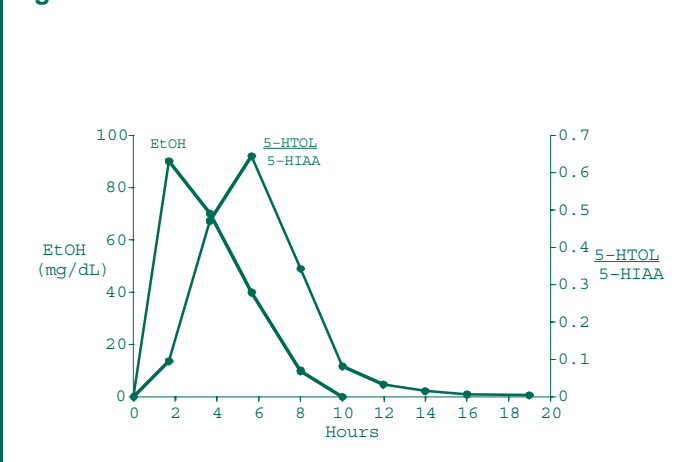
A better test would be for the presence of microorganisms. Their absence would preclude fermentation as the source of ethanol. However, their presence does not prove that the alcohol came from fermentation, it only raises the possibility. Some urine specimens spiked with glucose and microorganisms did not produce any appreciable ethanol even when stored at room temperature.

5-HTOL:5-HIAA ratio

Recently, an analytic technique has been developed that can resolve the issue of whether ethanol found in urine is the result of use or fermentation. It involves the determination of the ratio of urinary 5-hydroxytryptophol (5-HTOL) to 5-hydroxyindoleacetic acid (5-HIAA) (4, 5). Both are metabolites of the neurotransmitter serotonin, but 5-HIAA is by far the major metabolite (from oxidation of serotonin) with 5-HTOL present in much smaller amounts (<1%). After ethanol consumption, however, the normal oxidative pathway is competitively inhibited such that much more 5-HTOL is formed, which dramatically increases the ratio of 5-HTOL:5-HIAA (Figure 1). A cut-off of 15–20 pmol 5-HTOL/nmol 5-HIAA has been suggested as an indication of recent use of ethanol. Note that one study of apprehended drunk drivers demonstrated average ratios over 700 pmol/nmol.

There has also been concern about *in vivo* formation of ethanol prior to voiding. Several studies have addressed this possible source of ethanol, with interesting titles such as "Auto-Brewery Syndrome" and "Bladder Beer" (6). Although significant levels of ethanol have been measured in urine specimens from ethanol formation within the body, this occurs

Figure 1. Urine 5-HTOL:5-HIAA ratio vs. urine ethanol



only under extreme conditions of serious infection by microorganisms. Although it is recognized that there is normally ongoing low-level endogenous ethanol formation, typical concentrations are extremely low (1000 times lower than legal limits).

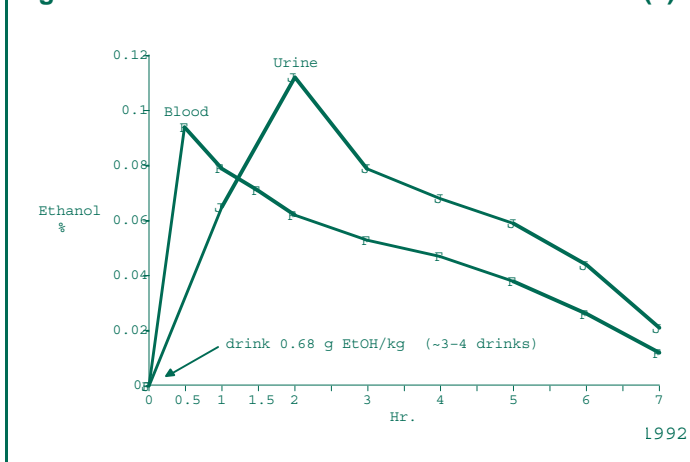
Finally, it should be noted that diffusion of alcohol through the bladder wall has been shown not to occur to any significant extent at concentrations found with drinking.

Urine: blood ratio

One of the challenges to the measurement of ethanol in urine specimens for forensic purposes is how well the results correlate to blood levels. Figure 2 shows a typical blood alcohol concentration (BAC) and corresponding urine alcohol concentration over time after dosing. Note that the urine level lags the blood level for the first urine collections and until the absorption maximum is passed. In the vast majority of drunk driving arrests, the subjects have been shown to be post-absorption.

Many studies demonstrate a urine: blood ratio of about 1.3:1. This reflects the greater water content of urine (about 95% water) relative to blood (about

Figure 2. Blood vs. urine concentration over time (8)



80% water). One of the most cited papers addressing the utility of determining blood alcohol concentrations from urine samples carefully reviewed the data from more than 20 published studies spanning over 50 years and found that the urine: blood ratio did not vary significantly from a 1.3:1 average except at very low alcohol concentrations, when first void samples were used (also as in autopsy cases), or when second samples were not collected soon (within 1 hour) after a first void (7).

However, in regulatory and legal rules, a ratio of 1.5:1 has been used to be conservative and give the benefit of any doubt to the subject. This is analogous to the common use of a 2100:1 blood:

breath ratio when 2300:1 would be more physiologically correct. To quote Biasotti and Valentine, "As applied in forensic science cases with cooperative subjects, *if the second 'sample' is used to determine the BAC using a ratio of 1.5:1 giving a BAC of 0.10% or greater, there is no scientific basis to believe that the actual sampling of blood would have produced a result less than 0.10%*" (italics in original).

Although some studies have demonstrated wide variability in the urine:blood ratio, an examination of the details of these studies reveals many of them are flawed. For example, some of these studies involve post-mortem analysis, which clearly has no relevance to the measurement of the ratio in living persons. Furthermore, studies involving initial voids have demonstrated a wider range of ratios than those involving analysis of second voids (after initial voiding of the bladder).

The limitation of a single first void is that one does not know over what time period the urine has collected in the bladder, although subjects who have been drinking would not be expected to maintain urine in their bladders for extended periods because of the diuretic effect of alcohol. Accordingly, in some studies even single-void specimens have demonstrated a urine:blood ratio of 1.3:1. Nonetheless, for a good correlation between urine and blood alcohol levels, two urine samples separated by about 20 to 30 minutes should be collected, with the first one usually discarded.

One study examined urine:blood ratios in 80 volunteers and 654 apprehended drivers (8). The author stated that when a second urine specimen is obtained within 30–60 minutes, a ratio of 1.35:1 gives a conservative estimate of the blood level existing during the time the urine was produced and accumulated in the bladder. However, the author noted that little confidence can be placed in a blood level calculated from a randomly timed urine specimen. Nonetheless, the author agreed that the urine:blood ratio can provide useful information on the absorption/elimination phase of the subject.

Although the author noted the wide range of ratios in his experiments as justification for not using urine for legal purposes, the marked deviations in the urine:blood ratio occurred with specimens obtained during absorption and at low blood alcohol levels. When the blood alcohol levels were in a legally relevant range and samples were collected properly, both the specimens from volunteers and the second-void specimens from drinking drivers fell in a narrow range for the urine:blood ratio. Another recent study of 40 drunk drivers also supported a urine:blood ratio of 1.3:1 with no effect from urine dilution (9).

Detection of any alcohol use

Although there may be challenges to the correlation between urine and blood alcohol levels, urine is nonetheless the most useful specimen to determine whether there has been any alcohol use. Because alcohol remains detectable in urine for slightly longer periods than in blood or breath (due to its presence in urine at higher concentrations than in blood and the possibility of urine being stored in the bladder slightly beyond the time when the blood and breath concentrations have declined to near zero), urine is the preferred specimen to determine if there has been any recent exposure to alcohol. Given an average elimination rate of 0.019% per hour, someone with a blood alcohol level of 0.1% would have detectable ethanol in blood for about 5 hours. Urine may provide an additional 1–2 hours of detection. Although this increased window may not seem like much in absolute terms, it is a significant increase over 5 hours.

Finally, it should be noted that excess water consumption does not affect urine ethanol levels, given that ethanol distributes quickly and evenly in total body water, so even an additional liter of fluid in a total body water volume of 40 liters will have a negligible effect (10).

Regulatory and legal issues

Of course, the most important legislation regarding alcohol testing comes from state drinking and driving statutes. At least 35 states specifically authorize urine alcohol measurements for driving-related offenses under implied consent statutes (using either a 1.3:1 or a 1.5:1 urine:blood ratio), and 11 states specify an alcohol concentration in urine for per se impairment statutes (that is, without a required conversion to a blood alcohol value). The Uniform Vehicle Code also authorizes urine alcohol testing. The validity of urine alcohol determinations is clearly supported by the weight of the above legislation.

In November 1989, the U.S. Department of Transportation (DOT) addressed the advantages and disadvantages of breath, blood, urine, and other techniques for alcohol testing (54 FR 46326). DOT noted that urine alcohol testing is less physically invasive than blood and requires the least on-site skill of all methods. In addition, because urine testing for controlled drugs is already common, instituting urine alcohol testing might require no additional personnel or on-site test equipment, which could be a significant cost advantage.

DOT acknowledged that a single urine sample can give evidence of recent prior use, although it

does not provide an accurate indication of current blood alcohol levels. However, a two-step collection procedure can indicate current alcohol levels. The DOT stated that although some might argue that the blood:urine correlation is insufficiently precise to be used as a measurement for higher cutoffs (such as 0.04 or 0.1 g/dL), the Federal Railroad Administration (FRA) authorized the use of urinalysis for alcohol testing using the conservative assumption that urine levels are 1.5 times greater than concurrent blood levels. Furthermore, the FRA drew attention to recent scientific literature endorsing such correlations.

In a December 1989 Final Rule (54 FR 53238), the FRA addressed objections to the use of urine alcohol analysis by stating that "reliance on the 'controversial' nature of applying the conversion factor, and the categorical statement that it is well known that urine alcohol cannot be converted with validity to BAC, were unsupported by citation to the literature, much of which the FRA has reviewed..... [S]tates continue to use urine (as an alternate or optional fluid) and have had success in the criminal arena, where the quantum of proof is quite high. FRA's recent review of statutes and case law indicated no adverse decisional law."

In spite of the foregoing scientific and legal validity, the FRA opted to delete any authority to perform for-cause alcohol testing on urine due to the pending rulemaking by the secretary of transportation cited above.

In October 1991, Congress passed the Omnibus Transportation Employee Testing Act, which provides for alcohol testing "in breath and body fluid samples, including urine and blood." However, urine is currently not used as a specimen for alcohol testing under these regulations.

Selected court rulings

Because of the many states' authorization of urine specimens in drunk driving offenses there are numerous cases addressing urine alcohol testing. A quick search of court cases using the term "urine alcohol" revealed 173 citations, although it is likely that many more cases actually addressed the use of urine for alcohol analysis. A review of the scientific literature on urine alcohol has also been published in a legal reference, evidence that the use and interpretation of urine alcohol results are issues in forensic and legal proceedings (11).

As previously mentioned, the first appellate court decision upholding the admissibility of alcohol test results in a drunk driving offense involved a urine alcohol test result (1). Also, in one of the first U.S. Supreme Court decisions on drug testing, post-

accident and reasonable-cause testing for alcohol and drugs using blood, breath, and urine were upheld (12). Among the challenged and upheld regulations was a provision for urine alcohol testing. So, in one sense, the validity of urine testing for alcohol has been acknowledged even at the Supreme Court level.

In a 1986 California appellate court case challenging the admissibility of urine alcohol test results, the court upheld urine alcohol testing noting there was nothing new about urine alcohol tests, that they had been used in California courts for 20 years, had been incorporated since 1966 in California's implied consent law statutes, and were carefully regulated to ensure accuracy. An additional challenge based on incomplete bladder voiding was denied because the court noted that the conversion factor based on empirical studies takes this into account (13).

Another example of judicial recognition of the validity of urine alcohol testing is a 1991 California court of appeals case in which the court found that "the implied consent law is tantamount to governmental acknowledgment a urine test is functionally equivalent to a blood test for evidentiary purposes with respect to a blood alcohol level" (14).

However, the issue of the urine: blood ratio did arise in a recent California court of appeals case which held that the cross-examination of the state criminalist regarding the variability in the urine: blood ratio must be allowed (15).

The issue of the possibility of fermentation also arose in a 1999 Wyoming Supreme Court case where a urine alcohol test result was 0.32% and there were claims of a false-positive result. The court held that there was a duty to properly collect and handle specimens, although there was no mention in the published opinion that the result was a false positive from fermentation (16).

The potential for fermentation in the urine from a diabetic occurred in a 1998 federal court of appeals case. A diabetic employee was discharged for a positive random urine alcohol test. The initial immunoassay on her urine specimen was 0.134% and a subsequent gas chromatography test was 0.258%. The laboratory did not use fluoride to prevent fermentation nor did the laboratory refrigerate the specimen. The employee had earlier settled her claims against her employer (17).

Summary

Although urine alcohol testing has not recently received the attention given to blood or breath, it is nonetheless a valuable tool to use in assessing alcohol exposure in a wide variety of settings. The analytical methods are accurate and precise, and the urine specimens are stable if properly handled. The

physiology of urine alcohol elimination is now well understood to allow for relatively straightforward urine collection procedures and interpretation of results. State legislation has clearly acknowledged all the foregoing, and has recognized the value of urine samples in drunk driving offenses. Other public and private entities have followed suit. Urine alcohol testing clearly has a significant role to play in society's efforts to address the problems of drug use.

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New On-Site Drug Testing Book Gives Views of Experts

On-Site Drug Testing is a new book in which scientists and forensic toxicologists critically evaluate the on-site devices currently available, their validation studies, and their use in a variety of settings. For each device, the expert contributors discuss its principles, materials and reagents, procedures and interpretation, and performance.

The tests covered include those for both therapeutic drugs (lipid-lowering medications, antithrombotic medications, and anticoagulant drugs) at the point of clinical care and drugs of abuse (alcohol, amphetamines, benzodiazepines, cannabinoids, cocaine, and opiates) in the workplace and criminal justice system.

The contributors address critical issues in sample collection and adulteration, and in program standards and legal requirements in workplace testing. The book contains 18 chapters, each written by one or more well-known experts, and is designed to provide a firm basis for choosing the best test devices and techniques for the reader's purposes.

Reviews of the book have been positive, with *Medical Review Officer Update* noting that as "the only current reference book devoted to this topic" it will be "most useful" for forensic toxicologists.

The editors of *On-Site Drug Testing* are Amanda J. Jenkins of the Cuyahoga County (Ohio) coroner's office and Bruce A. Goldberger of the University of Florida College of Medicine in Gainesville. The 304-page hardcover, list price \$89.50, is published by Humana Press; information is available at www.humanapress.com.

POC Oxycodone Test Cleared

Rapid One OXY, a point-of-care urine assay for oxycodone made by American Bio Medica Corp. of Kinderhook, New York, has received Food and Drug Administration clearance. The assay, which provides results in minutes from a urine sample, is designed to detect oxycodone at low levels and does not detect other opiates at these concentrations.

Labs Need to Set Computer Confidentiality Policies

By Wayne Markus

Confidentiality has long been an ethical requirement in clinical medicine, religious counseling, and forensic urine drug testing (FUDT). The individual has a right to expect that private information will not be passed on without express permission or an overwhelming reason such as protection of another (such as when a man tells his pastor that he is going to kill his wife and family). The ethical requirement of maintaining confidentiality of patient medical information, including laboratory test results, is no different today than it has been for many years.

Hospitals and medical care facilities nearly always have a confidentiality policy for their employees. Many require employees to sign confidentiality statements. A breach of the confidentiality policy can often lead to termination of the employee.

There has been greater emphasis on confidentiality in FUDT since the advent of the FUDT Accreditation Program and the Substance Abuse and Mental Health Services Administration's program for workplace drug testing. This may be in part because workplace drug testing does not fall under the usual umbrella of clinical medicine, and thus not necessarily under the same ethics.

Electronic standards

The Health Insurance Portability and Accountability Act of 1996 (HIPAA) and the resultant proposed security and electronic signature standards are affecting medical-care providers. Providers are struggling to comply with the regulations' administrative procedures to guard data integrity, confidentiality, and availability. Application of the rules is not always straightforward. At one extreme are the "HIPAA Hypers" and at the other are those whose attitude is much too relaxed. Although the HIPAA rules do not apply to workplace drug testing, many FUDT laboratories also do clinical testing, and thus may be required to comply. Even the strictly FUDT laboratory may find the rules a valuable resource.

Today nearly all laboratories of any size have a laboratory information system (LIS) of one type or another. An LIS may contain standard operating procedures (SOPs), policies, demographics, scanned information, accessioning information, chain-of-custody records, raw and interpreted laboratory data, final reports, medical review officer (MRO) data, and billing information. It may have remote connections to collection sites and for reporting.

Computer processing

Much confidential information may be processed and stored in a laboratory information system and travel via telephone lines, microwaves, fiber optics, and communication satellites. Maintaining confidentiality requires more in the information age than in the days of handwritten reports delivered to the health-care provider. Third-party payer and managed-care systems add to the complexity of maintaining confidentiality.

Policies and SOPs regarding confidentiality and security of computer data must be documented in either hard-copy or electronic form. They must be sufficiently complete and current. There must be documentation of approval by the scientific director. There must be documentation of training of personnel who have access to computer data or who have access to confidential information.

The computer should be located in an area of controlled access or be protected to prevent unauthorized access to the hardware. It may be within the FUDT laboratory or in another secure location. It may even be in another facility or state.

Access policies

There must be a policy and procedure on access to the computer approved by the scientific director. The individuals responsible for determining levels of access must be defined, with the levels of access tailored to the LIS. The levels might include updating and modifying the system, programming, archiving, data entry, data retrieval, reporting, modification of reports, and billing.

There should be a policy on password access codes and on unauthorized sharing of passwords with others, with documentation that each employee with a password has been informed of the policy.

Access should be limited to those who need it. Some systems allow certain job titles to have access to functions and data across departments. For example, an employee in hematology might have access to similar data and functions in the FUDT laboratory. Employees who have no need for access to FUDT data should not have it. This is less of a problem when the computer is dedicated to the FUDT department or in the freestanding FUDT laboratory.

There must be records of who has the various levels of access and when it was granted. There must be a policy on retention of access records.

It is desirable to have a record of individual log-ons and attempted access to the computer. Security testing of the system should be documented.

Although they are directly related to confidentiality, there should be policies and procedures for vi-

rus protection, disaster recovery, and backup, recovery, and archiving of programs and data.

Remote access

If the computer can be accessed remotely, via modem or Internet, there must be policies and procedures on how access is controlled and limited. Electronic reporting over modem, remote printers, facsimile, mobile telephone, personal data assistants, intranet, or Internet requires policies and procedures on its usage. Contact persons at the remote sites and recommendations on security and confidentiality may need to be tailored to the site and system. Ultimately, confidentiality at the remote site is the responsibility of both the laboratory and the remote site. If the remote site has access to the host computer, policies and procedures that address passwords and confidentiality must be documented.

Whether the LIS is off site, in another laboratory, or in another state, the requirements are the same. There must be copies of appropriate policies, procedures, and other records in the laboratory. The scientific director must approve them. If the host computer is in another FUDT-accredited laboratory, the inspection team will not be required to do an on-site inspection of the computer system. The host computer will be included in the inspection of that facility. Both laboratories must have copies of the appropriate policies and procedures as well as records specific to the facility inspected so that the checklist questions can be adequately reviewed.

Laboratory information systems are becoming far more sophisticated. Most laboratories use a commercial package and have little control over the performance of many functions. The laboratory is not expected to understand programming code, but the laboratory director must ascertain an LIS' compliance with requirements for confidentiality and other functions before it is placed in service.

Wayne Markus, MD, is chair of the Toxicology Resource Committee of the College of American Pathologists and a pathologist with Physicians Laboratory in Omaha, Nebraska.

Correction

The March *CFTN* carried a notice of a new Substance Abuse and Mental Health Services Administration (SAMHSA) website at www.drugfreeworkplace.com. A sharp-eyed reader pointed out that this address leads not to a government site, but to one run by National Medical Review.

The SAMSHA site can be found at www.drugfreeworkplace.gov or <http://workplace.samhsa.gov>.

Drugs in Water Pose an Emerging Water Quality Issue

By Timothy Chapman

Pharmaceutical residues originating from treated sewage effluent occur widely in European surface, ground, and drinking water. Monitoring programs in the United States, Canada, and Brazil have also detected drug residues in ambient water. The drugs detected include analgesics, cardiovascular drugs, anti-septics, chemotherapy agents, antibiotics, and hormones. The U. S. Geological Survey (USGS) recently listed numerous drugs as target compounds to monitor as "emerging contaminants" in U.S. streams (Table 1).

Table 1. USGS target compounds for national reconnaissance of contaminants in streams

Metformin (antidiabetic)
Cimetidine (antacid)
Ranitidine (antacid)
Enalaprilat (antihypertensive)
Digoxin (cardiovascular)
Diltiazem (antihypertensive)
Fluoxetine (antidepressant)
Paroxetine (antidepressant)
Warfarin (anticoagulant)
Salbutamol (antiasthmatic)
Gemfibrozil (antihyperlipidemic)
Dehydronifedipine (antianginal metabolite)
Digoxigenin (digoxin metabolite)
Acetaminophen (non-steroidal anti-inflammatory)
Ibuprofen (non-steroidal anti-inflammatory)
Codeine (analgesic)
Caffeine (stimulant)
1,7-Dimethylxanthine (caffeine metabolite)

Conventional sewage-treatment technologies vary greatly in their ability to eliminate drug residues. Documented ecological impacts of these residues in ambient water include the development of antibiotic-resistant bacteria in rivers and birds, and sexual disruption of fish exposed to estrogenic chemicals.

Drug concentrations in the parts per billion range have been reported in groundwater samples. Several drugs, including propoxyphene, phenytoin, amitriptyline and caffeine are present in New Mexico water samples at parts per trillion concentrations (Table 2). Water samples were taken from sewage effluent outfalls, surface water receiving sewage, and public drinking-water systems served by surface water or by groundwater that may contain treated sewage.

Table 2. Maximum concentrations of selected drug residues detected in water

In treated sewage	In surface water
Propoxyphene: 820 ng/L	Amitriptyline: 30 ng/L
Phenytoin: 300 ng/L	Caffeine: 200 ng/L
Caffeine: 1000 ng/L	Ethinyl estradiol: 10 ng/L
Amitriptyline: 30 ng/L	

Potential contamination of water resources with pharmaceutical drugs is an area of growing concern. The toxicological significance of long-term exposure at low levels is poorly understood by public health experts. Projects like the Toxic Substances Hydrology Program of the USGS hope to investigate the significance of this type of exposure to drugs, as well as other emerging contaminants such as antioxidants, fire retardants, and plasticizers.

Suggested Reading

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New On-Line Toxicology Continuing Education

The AACC has posted new on-line presentations on volatile alcohols, glycols, and clinical alcohol testing.

There are four presentations, and users can earn up to four hours of continuing education credit (ACCENT or CME), all free of cost.

The website even features periodic live interactive sessions in which questions can be sent via e-mail with presenters sending back immediate replies.

To participate in this brand new program, log on to: www.aacc.org/symposia/clintox/alcohols/default.stm.

The purpose of *Clinical & Forensic Toxicology News* is to provide practical and timely information on the clinical, forensic, technical, and regulatory issues faced by toxicology laboratories.

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