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Ingestion of Toxic Alcohols and Glycols: An Ongoing Diagnostic and Analytic Challenge

By Heather R. Greene and Matthew D. Krasowski, MD, PhD

The ingestion of toxic alcohols and glycols, including ethylene glycol, isopropanol, and methanol, remains a significant public health problem (1–3). These compounds are easily obtained from widely available and inexpensive household and industrial products. Similar to ethanol, the toxic alcohols and glycols can cause central nervous system (CNS) and respiratory depression that may be particularly dangerous when combined with other CNS depressants, such as benzodiazepines and opioids. Ethylene glycol and methanol can also produce organ damage and potentially life-threatening complications from the toxicity of their metabolites. Consequently, such ingestions should be treated as medical emergencies. Another glycol less commonly associated with symptomatic toxicity is propylene glycol, a compound found in some automobile antifreezes and also used as a diluent or excipient in some medications. In addition, propylene glycol can also produce analytic interference with enzymatic assays for ethylene glycol, as will be discussed below. This article will first review the specific toxic alcohols and glycols and then discuss analytical methods and challenges.

Ethylene Glycol

Ethylene glycol is the primary component of most marketed formulations of automobile antifreeze (1, 4). Propylene glycol is the main component of alternative antifreezes that do not contain ethylene glycol. Ethylene glycol has a sweet taste, a factor that may contribute to accidental ingestion by children and pets. The first step in the metabolism of ethylene glycol is to glycolaldehyde by alcohol dehydrogenase (ADH), the enzyme that also catalyzes the initial rate-limiting step in the metabolism of ethanol and methanol (5). Glycolaldehyde is subsequently converted to glycolic acid, a metabolite that can produce cardiac, respiratory, and neurologic toxicity. Glycolic acid is also the main factor producing the increased anion gap and metabolic acidosis commonly seen in ethylene glycol ingestions, especially those that are not treated quickly. The conversion of glycolaldehyde to glyoxylic acid and then to oxalic acid can result in calcium oxalate crystal formation in the renal tubules approximately 24 to 72 h after ethylene glycol ingestion. Damage from oxalate crystals can produce significant and potentially permanent renal injury (1, 4). The metabolism pathway of ethylene glycol is summarized in Fig. 1.

Methanol

Methanol (methyl alcohol, "wood alcohol") is found in a variety of products, including automobile windshield fluid and industrial cleaning supplies. Methanol ingestion is overall less common than ethylene glycol ingestion (2). Improperly produced "bootleg" alcohol contaminated with methanol has resulted in outbreaks of methanol toxicity (6). Methanol is metabolized first by ADH to formaldehyde and then to formic acid (7). Formic acid can produce permanent injury to the optic nerves, leading to blindness. The metabolism pathway of methanol is summarized in Fig. 2.

Isopropanol

Isopropanol ("rubbing alcohol") is found in a variety of products. The misperception that rubbing

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alcohol contains ethanol and not isopropanol can lead to accidental ingestions (3). Rubbing alcohol may also be intentionally used as an alternative intoxicant if ethanol is not available. The presence of isopropanol within medical facilities may result in ingestion by patients if the bottles are not stored securely. Although isopropanol can produce significant CNS and respiratory depression, isopropanol ingestion is generally less harmful than ethylene glycol or methanol owing to absence of toxic metabolites. Isopropanol is metabolized to acetone, which is a normal component of human metabolism. Medical management of isopropanol ingestions is supportive, and there is no specific antidote used to treat isopropanol intoxication.

Propylene Glycol

Although similar in name and molecular structure to ethylene glycol, propylene glycol is considered less toxic (8, 9). Propylene glycol is the constituent of some antifreezes that may be marketed as "less toxic" or more "environmentally safe" alternatives to those containing ethylene glycol. Propylene glycol may be present in cosmetics, foods, tobacco products (including electronic cigarette solutions), and pharmaceuticals. As a solvent that can dissolve hydrophobic substances even in the presence of water, propylene glycol is used as a diluent or an excipient for medications including activated charcoal and poorly water-soluble intravenous medications such as lorazepam and diazepam (8, 9). Iatrogenic propylene glycol toxicity may result from continued use of intravenous medications dissolved in propylene glycol. The most extensive literature on this topic has been in critical care settings, where there is extended use of intravenous lorazepam for intubation or other clinical reasons (10, 11). There are also case reports of propylene glycol toxicity following ingestion of propylene glycol-based antifreeze products (12). Propylene glycol is metabolized to both L- and D-lactate; consequently, D-lactic acidosis may be observed in propylene glycol poisoning. Similar to isopropanol, medical management of propylene glycol ingestions is supportive, with no specific antidote available (8, 9).

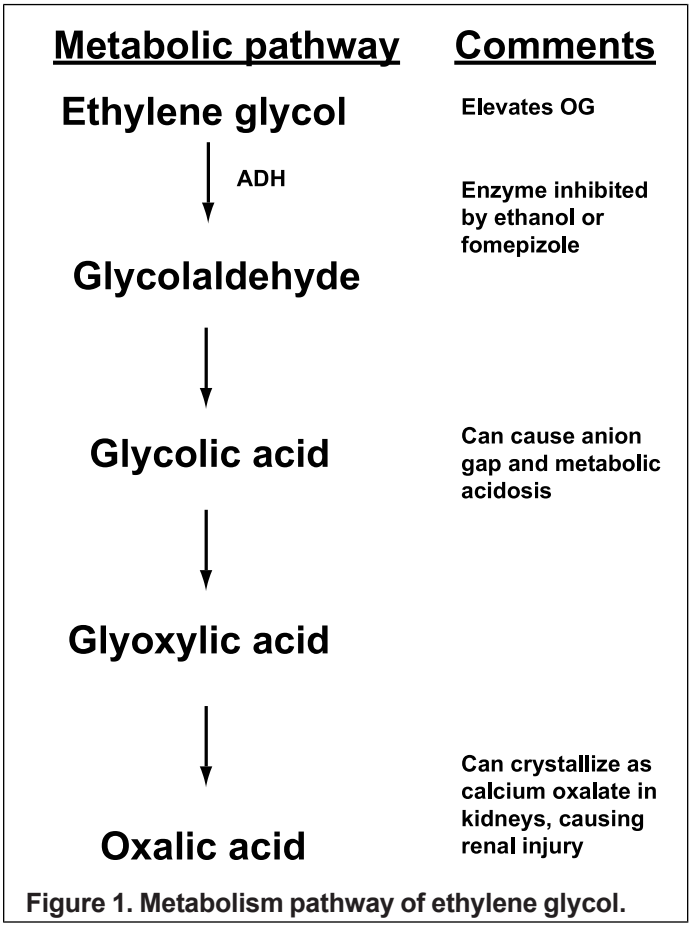

Figure 1. Metabolism pathway of ethylene glycol.

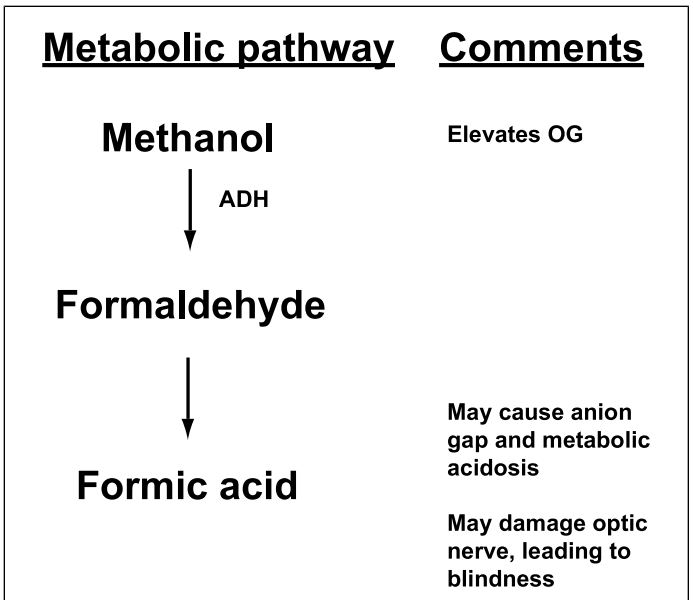

Figure 2. Metabolism pathway of methanol.

Treatment of Toxic Alcohols

Antidote therapy for ethylene glycol and methanol intoxication focuses on inhibition of ADH, which catalyzes the rate-limiting enzymatic step in the metabolism of both compounds (2). The goal is to prevent the formation of toxic metabolites, such as glycolic acid or oxalic acid for ethylene glycol or formic acid for methanol. The parent drug (ethylene glycol or methanol) can then be cleared by the

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kidneys. Fomepizole (4-methylpyrazole), a specific ADH inhibitor, has emerged as a specific treatment for methanol and ethylene glycol poisoning (5, 7). The expense of fomepizole can be a limitation, especially for hospitals that rarely see ingestions (13). This may lead to creative solutions, such as a group of hospitals sharing supply of this drug.

An alternative antidote choice for ethylene glycol or methanol poisoning is to use ethanol itself to inhibit ADH; this may especially be an option if the expense of fomepizole is prohibitive (2). One risk of using ethanol is that this treatment may compound the CNS and respiratory depression caused by the ethylene glycol or methanol. Thus, mortality and adverse events are higher in patients receiving ethanol compared with fomepizole as antidote. In some patients, coingestion of ethanol may fortuitously limit the metabolism of ethylene glycol and methanol to toxic metabolites. However, once metabolites have been generated, fomepizole and ethanol have less benefit, and organ damage from metabolites may occur. If too much time has passed, hemodialysis, an invasive procedure, is required to remove these other compounds (2). Hemodialysis is recommended for ingestions involving very increased levels of methanol and/or ethylene glycol, deteriorating vital signs despite supportive care, vision disturbance with methanol, or renal failure.

Diagnostic Challenges

Toxic alcohol and glycol ingestions can be difficult to diagnose because of the nonspecific signs and symptoms (1–3, 14). People who have ingested these compounds often cannot provide an adequate history, and history may be available only from other witnesses. It is critical to diagnose toxic alcohol ingestions quickly, particularly when they involve methanol or ethylene glycol, because the efficacy of treatment depends on how much time has elapsed. Basic chemistry tests and arterial blood gas analysis can be performed quickly to provide information that can guide treatment. The anion gap may also provide valuable clinical information (15, 16). Ethylene glycol and methanol ingestions are known causes of anion gap metabolic acidosis owing to the production of metabolites such as glycolic acid and formic acid.

Analytical Methods for Direct Measurement of the Toxic Alcohols and Glycols

Gas chromatography, alone or together with mass spectrometry (GC/MS), is the gold standard for detection and measurement of toxic alcohols and glycols in blood samples (1–3, 16). Quantitative serum/plasma concentrations guide treatment decisions and, depending on clinical circumstance, may be part of the clinical decisions on whether to stop or continue antidotal therapy or hemodialysis. However, gas chromatography or GC/MS is time- and labor-intensive. As a result, many facilities do not perform gas chromatography or GC/MS analysis in-house, and the turnaround time to have it performed at a remote regional or reference laboratory is often too long for time-sensitive diagnostic and treatment decisions (4, 17, 18). This may lead to use of antidote and/or hemodialysis as empiric therapy in cases for which ingestion is not certain based on initial history and clinical signs or to missing the diagnosis altogether when severe toxicity may still be preventable.

One alternative method that has emerged for analysis of ethylene glycol is the use of enzymatic assays that can run on standard clinical chemistry analyzers to determine quantitative ethylene glycol concentrations (19–21). Ethylene glycol enzymatic assays have been marketed for veterinary medicine, given that household pets and other animals may ingest antifreeze or other ethylene glycol-containing products (22). To our knowledge, no ethylene glycol enzymatic assays are approved to date by the Food and Drug Administration (FDA). Thus, clinical laboratories interested in performing these assays need to perform validation as laboratory-developed tests, with several laboratories publishing methods for different chemistry analyzers (19–21).

The major limitation of rapid ethylene glycol assays is interference by structurally related compounds that may be found in patient specimens, notably propylene glycol and 2,3-butanediol (19, 21). The use of activated charcoal and intravenous medications containing propylene glycol means that patients may have been exposed to propylene glycol before diagnostic workup for ethylene glycol begins. In a study previously published from our institution (an academic medical center), propylene glycol interference was detected in slightly more than 10% of samples submitted for rapid ethylene glycol analysis, reflecting a patient population receiving emergency care and associated medications (21). The presence of propylene glycol can be inferred from examination of the reaction rate kinetics of the ethylene glycol enzymatic assays. A national reference laboratory reported that method optimization on the Beckman Coulter Olympus AU400 platform was able to bypass propylene glycol interference, although this may be technically challenging on other chemistry instruments (19).

The clinical relevance of interference by 2,3-butanediol is less certain. In vitro analysis indicates this compound interferes with enzymatic ethylene glycol assays and also with some gas chromatography methods for ethylene glycol (19, 23). Published literature from the 1980s demonstrated that 2,3-butanediol is increased in individuals with chronic alcohol use (24, 25), but there have been few published investigations related to this compound in human specimens since then. 2,3-Butanediol analysis is not commonly performed for clinical

purposes. Thus, it is open to future investigation to ascertain the distribution and quantitative levels of 2,3-butanediol in patient populations and how this may affect ethylene glycol analysis.

Use of Osmolal Gap for the Toxic Alcohols and Glycols

The presence of ethylene glycol, isopropanol, or methanol in serum or plasma can often be determined and monitored by measuring the osmolal gap (OG) (15, 16, 26, 27). However, other compounds originating from endogenous or exogenous sources, such as propylene glycol and acetone, can also increase the OG (8–11, 28). An increased OG suggests the presence of osmotically active substances in addition to typical endogenous contributors (15, 16, 29, 30). This may occur with toxic alcohol or glycol ingestions or with a variety of pathologic conditions, including alcoholic ketoacidosis, diabetic ketoacidosis, renal failure, and shock. The infusion of mannitol (osmotic diuretic) for conditions such as increased intracranial pressure may also increase the OG. In cases of toxic alcohol or glycol ingestion, the reduction of an increased OG during treatment can estimate elimination of the toxicant from the body.

The OG is determined by first measuring serum/plasma osmolality ("measured osmolality") on an osmometer, ideally using freezing point depression. A "calculated osmolality" is then determined using common laboratory tests such as blood urea nitrogen, glucose, and sodium to estimate the osmolality from major endogenous components of serum/plasma. If ethanol is present, its estimated contribution to osmolality can also be included in the calculated osmolality. The difference between measured and calculated osmolality is referred to as the OG. There is a complicated body of literature on the myriad formulae that have been proposed for estimating serum/plasma osmolality and also of how actual patient specimens deviate from ideal conditions that are assumed in the OG calculation (27, 29–31).

Conversion factors can be used to estimate specific concentrations of ethylene glycol, isopropanol, methanol, acetone, and propylene glycol from OG. Standard conversion factors for estimating toxic alcohol and acetone concentrations in milligrams per deciliter by multiplying OG by the conversion factor are ethylene glycol, 6.2; isopropanol, 6.0; methanol, 3.2; acetone, 5.8; and propylene glycol, 7.6 (3, 4, 17). A recently published study by our group shows that these conversion factors provide estimates of toxic alcohol or glycol concentrations in patient samples that correlate reasonably well with direct measurement by gas chromatography and/or rapid ethylene glycol enzymatic assay (32). It is important to note that these conversion factors assume minimal or no contribution to OG by metabolites. This assumption holds fairly well for ethylene glycol and methanol owing to their conversion to metabolites (e.g., glycolic acid, formic acid) that dissociate in the blood. As discussed above, the metabolites of ethylene glycol and methanol contribute to metabolic acidosis and increased anion gap. However, although they may increase serum osmolality, the impact of these anions on OG is nullified by the inclusion of serum/plasma Na^+ concentration multiplied by a factor of 2 (or similar cofactor) in calculated osmolality equations (1).

Thus, ethylene glycol and methanol can have a classic pattern in which they first produce an increased OG ("early" ingestion). An anion gap, metabolic acidosis develops later if no treatment is initiated ("late" ingestion). There may be an intermediate period during which an increased OG and anion gap are both present. Isopropanol is more complicated, given that both isopropanol and its metabolite acetone (which does not dissociate in serum/plasma to any appreciable degree) can contribute significantly to osmolality and OG (3). The contribution of ethanol to osmolality and OG is also complicated owing to unmeasured metabolites of ethanol or pathophysiologic complications, such as alcoholic ketoacidosis, that may also affect OG (2, 30). It is also important to note that some ingestions may not increase OG or anion gap (15, 16). For example, a toxic level of 20 mg/dL ethylene glycol will contribute only about 3 units to the OG.

Previous studies have examined the relationship between methanol or ethylene glycol serum/plasma concentrations estimated by OG (using conversion factors) and those directly measured by gas chromatography and found these are best represented by a linear model (26). Other studies have looked at propylene glycol concentration and toxicity in the context of lorazepam infusion (10, 28, 33). In general, studies of propylene glycol tend to demonstrate more widely distributed data for the relationship between propylene glycol concentration and OG but, overall, a linear relationship. Data on the relationship of isopropanol ingestion to OG have been sparse in the literature. Our recent study found that OG measured from actual isopropanol ingestions correlated linearly with actual concentrations of isopropanol and acetone determined by gas chromatography (32). It also appears that the isopropanol plus acetone concentration estimated from the OG is generally a good approximation of the actual combined concentration of these species. This implies that a conversion factor of 5.9, the average of the individual conversion factors for acetone (5.8) and isopropanol (6.0), is a reasonable approximation.

Discussion

The ingestion of toxic alcohols and glycols continues to be a diagnostic and laboratory challenge. A fundamental barrier is that the gold standard

methodology (gas chromatography or GC/MS) is not widely available for rapid turnaround times in many locations. Thus, diagnosis and management typically rely on clinical history, signs, symptoms, and indirect laboratory methods such as OG and anion gap. A limited number of clinical laboratories have adapted veterinary ethylene glycol enzymatic assays for analysis of human samples, and method validation for these assays on multiple chemistry platforms has been reported in the published literature. However, the lack of FDA-cleared assays is likely a barrier to many laboratories considering this approach. The interferences of ethylene glycol enzymatic assays with structurally similar compounds (e.g., propylene glycol, 2,3-butanediol) are also a practical limitation to contend with. To our knowledge, enzymatic methods for specific analysis of methanol in human samples have not been reported. In contrast to ethylene glycol, methanol is a much less common poisoning in animals, reducing the market for a veterinary methanol assay that could be adapted for human samples. Clinical laboratories can assist clinical teams by knowing the limitations and strengths of OG, anion gap, blood gas analysis, and other laboratory testing used in diagnosis and management of toxic alcohol and glycol ingestions.

Learning Objectives

After reading this article, the reader will be able to describe the analytical methods for specifically detecting and measuring serum/plasma concentrations of ethylene glycol, isopropanol, methanol, and propylene glycol, and identify the role and limitations of the osmolal gap in the diagnosis and management of toxic alcohols and glycols.

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Fentanyl Test Strip—An Unconventional Tool in the Fight Against the Opioid Overdose Epidemic

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The opioid overdose epidemic is one of the most remarkable public health challenges of the 2010s in the US. In 2017, the number of drug overdose deaths reported in the US was 70 237, with opioids accounting for about 47 600 of these deaths (1). Of the 47 600 opioid-related overdose deaths, 28 000 were attributed to synthetic opioids (excluding methadone). The steady increase in opioid-related deaths between 2013 and 2017 was primarily driven by the adulteration of common street drugs like heroin, cocaine, and methamphetamine with illicitly manufactured fentanyl and fentanyl analogs (e.g., carfentanil, sufentanil, furanylfentanyl, and acetyl fentanyl) (2). Data reported by the National Forensic Laboratory Information System show an increase of >5000% in drug submissions that tested positive for fentanyl between 2013 and 2017 (3).

Fentanyl is a phenylpiperidine-derived opioid receptor agonist that has about 100 times higher analgesic activity compared with morphine owing to its enhanced lipophilicity and its ability to rapidly cross the blood–brain barrier (4). Since its introduction as an intravenous anesthetic in the 1960s, several other pharmaceutical formulations of fentanyl, including oral transmucosal lozenges, sublingual tablet, transdermal patches, nasal spray, and buccal tablets, have been used in medical practice for pain management. The bioavailability of fentanyl formulations ranges from 50% for oral transmucosal lozenges (5) to 76% for sublingual sprays (6). The onset of action for fentanyl is almost immediate when administered intravenously; however, its duration of action is short (<2 h). Fentanyl has a mean volume of distribution of 4 L/kg with a pKa of 8.4 and an elimination half-life of 5 to 12 h (6). Approximately 80% to 85% of fentanyl in circulation is bound to plasma proteins, primarily albumin and α1-acid glycoprotein (7). The primary product of fentanyl metabolism is norfentanyl, which is an inactive metabolite produced through *N*-dealkylation by the cytochrome P450 liver enzymes (CYP3A4 and CYP3A5) and accounts for >99% of fentanyl metabolism (8, 9). Other minor fentanyl metabolites include despropionylfentanyl and hydroxyfentanyl (8, 9).

Similar to other opioid receptor agonists, the analgesic effect of fentanyl stems from its ability to activate the μ-opioid receptor and inhibit neural excitability responsible for pain transmission in the central nervous system (10). In addition to its analgesic effect, fentanyl also induces intense euphoric effect and feelings of pleasure by stimulating the brain's dopamine reward pathway. This contributes to its high potential for abuse and increased risk of dependence and addiction. Although the median lethal dose (LD_{50}) of fentanyl in humans is unknown, blood fentanyl concentrations as low as 5 ng/mL have been reported in overdose deaths involving only fentanyl (11). The associated effects of fentanyl overdose include respiratory depression, skeletal muscle flaccidity, and hypotension, which can often culminate in coma and death.

Legal fentanyl prescriptions remain essential for the treatment of some patients (e.g., opioid-tolerant cancer patients with breakthrough pain) when used under the guidance of a licensed healthcare professional. However, illicitly manufactured fentanyl and fentanyl analogs have become popular among drug traffickers as a cheap and potent lacing

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agent in counterfeit prescription pills and common street drugs. Although some street drug users may seek out fentanyl-containing drugs because of their enhanced potency, the majority of street drug users are unaware of the content and purity of the illicit drugs they purchase and, therefore, are at great risk for overdose from fentanyl-contaminated drugs. In an effort to minimize accidental overdose from street drugs adulterated with illicitly manufactured fentanyl, harm reduction initiatives such as naloxone distribution programs and supervised consumption facilities have been implemented by public health organizations. More recently, the concept of drug checking using fentanyl test strips has also been proposed as a strategy for reduction of opioid overdose death rates (12–14).

The fundamental idea behind drug checking using fentanyl test strips is that an individual about to use illicitly purchased drugs would first analyze the drug sample to determine whether it is adulterated with fentanyl. If fentanyl is detected, then it is hoped the individual would recognize the risk of potential overdose and change drug use behavior. For example, the individual may decide not to use the drug, use the drug in the presence of someone with access to naloxone, use a lower dose of the drug, inject an initial tester shot of a liquid formulation to gauge the potency of the drug, reduce rate of drug injection by pushing the syringe plunger very slowly, or snort the drug instead of intravenous injection (12–14). The use of fentanyl test strips is increasingly gaining popularity and is being actively advocated for by public health organizations like DanceSafe, Harm Reduction Coalition, and St. Ann's Corner of Harm Reduction. Additionally, there is evidence of high willingness to use fentanyl test strips by people who use drugs (14–16).

The fentanyl test strips are lateral flow chromatographic immunoassay strips originally designed for the qualitative detection of fentanyl in urine. The Rapid Response™ Fentanyl Forensic Test Kit manufactured by BTNX is the most popular brand of fentanyl test strip distributed by harm reduction organizations and has a detection limit of 0.2 μg/mL with an accuracy of >98% (17). The BTNX fentanyl test strip is a competitive immunoassay strip that produces a colored line in the test region only in the absence of fentanyl-containing samples owing to unsaturated antibody binding sites. Therefore, appearance of 1 colored band (in the control region) indicates a fentanyl-positive specimen, whereas 2 colored bands (one in the control region and another in the test region) indicate a fentanyl-negative specimen, as shown in Fig. 1. A recent study comparing the BTNX fentanyl test strip with 2 other drug-checking technologies (Raman spectroscopy using a TruNarc machine and Fourier-transform infrared spectroscopy using a Bruker Alpha machine) found that the fentanyl test strip had the lowest detection limit (0.13 μg/mL) and the highest sensitivity (96%–100%) and specificity (90%–98%) (16). The BTNX fentanyl test strip has good cross-reactivity with some fentanyl analogs like carfentanil, acetyl fentanyl, butyryl fentanyl, 3-methyl fentanyl, ocfentanil, remifentanil, sufentanil, furanylfentanyl, and valeryl fentanyl; however, cross-reactivity with other synthetic opioids like U-47700 have not been determined (17). Additionally, it is worth mentioning that at the time of writing this article, BTNX fentanyl test strips have not been approved by the US Food and Drug Administration.

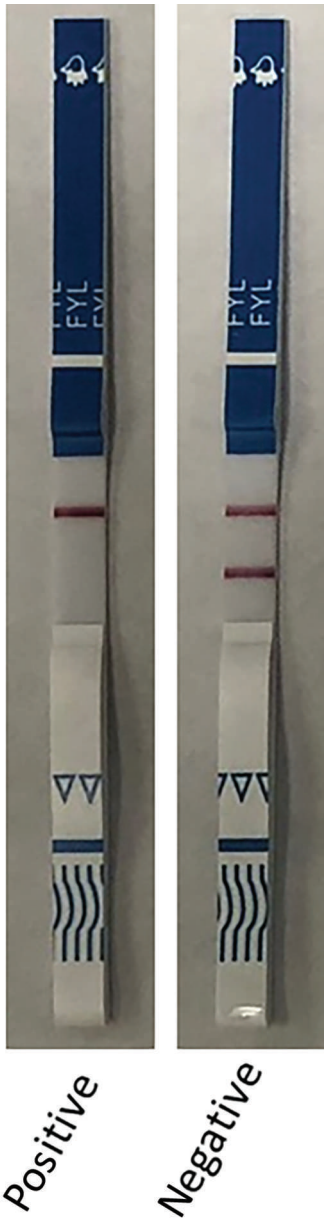

Figure 1. A picture of a Rapid Response™ fentanyl test strip showing a positive result and negative result.

The concept of drug checking using fentanyl test strips may seem like a worthy initiative to

mitigate the increasing rate of opioid overdose deaths. However, careful consideration must be given to the limitations of fentanyl test strips and their associated consequences. Street drugs and counterfeit prescription pills that are adulterated with illicitly manufactured fentanyl are subject to varying preparation practices, which can change the homogeneity, solubility, and pH of the final formulation (18). These factors may compromise the accuracy of the test strip, leading to false-negative results that would create a false sense of security and predispose the drug user to possible overdose or death. The majority of fentanyl test strip pilot studies have been conducted in supervised settings where trained personnel either perform the testing or provide proper guidance and instructions (13, 14, 16). In an unsupervised environment, it is likely that there will be varying degrees of competency on how the strips are used and interpreted by people who use drugs, especially when they are experiencing withdrawal symptoms and are desperate to stave it off. Additionally, fentanyl test strips do not give information about the concentration of fentanyl or fentanyl analog contained in adulterated drugs. It is unlikely that a drug user will seek out additional testing to quantify and characterize the fentanyl contamination in their illicitly purchased drug. Most importantly, it should be emphasized that even if unadulterated, heroin, oxycodone, and other commonly abused opioids can still lead to overdose and death.

Drug addiction and substance use disorder are biochemical changes in the brain that induce intense drug craving and compulsive use, despite harmful consequences, in an effort to avoid withdrawal symptoms. Although drug checking using fentanyl test strips may potentially reduce unintentional overdose from fentanyl-adulterated drugs, it does not provide a solution to drug addiction. One can make the argument that fentanyl test strips might encourage people to continue using illegal drugs. Hence, it is important that fentanyl test strips are implemented in the context of other programs designed to provide treatment for substance use disorder and help provide a path of recovery for people addicted to drugs (16).

Learning Objectives

After reading this article, the reader will be able to describe the pharmacology of fentanyl and the contribution of illicitly manufactured fentanyl to opioid overdose deaths in the US. The reader will also be able to describe how a fentanyl test strip works, its limitations, and its proposed use as a tool for preventing opioid overdose.

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Oral Fluid Mandatory Guidelines Update

By Claudia Henemyre, PhD and Jean Chambers, BS

The Department of Health and Human Services (HHS) published the final Mandatory Guidelines for Federal Workplace Drug Testing Programs using Oral Fluid (OFMG) in the Federal Register (42 CFR) (1) on October 25, 2019, with an effective date of January 1, 2020. The OFMG provides the scientific and technical guidelines to permit federal executive branch agencies to add oral fluid testing to their workplace drug testing programs. The expectation is enhanced flexibility and versatility through agency authorization to collect oral fluid and increased collection site possibilities. Observed oral fluid collections should also reduce invalid results. The transition to oral fluid is predicted to take 4 years, eventually accounting for 25% to 30% of federal agency drug testing with the same level of confidence as urine testing. It should be noted that the Department of Transportation has yet to publish a proposed rule for oral fluid testing; the current guidelines apply only to federal agency employees.

The main topics covered by the OFMG include oral fluid collection devices and procedures, initial drug test analytes and methods, confirmatory drug test analytes and methods, procedures for review by a medical review officer (MRO), and requirements for federal agency actions, to include laboratory certification.

HHS will revise the Federal Custody and Control Form and will issue an Oral Fluid Specimen Collection Handbook to provide guidance on proper collection and transport of oral fluid specimens. Each oral fluid specimen is collected as a split specimen. The options are as follows: (*a*) simultaneous collections using 2 separate devices, (*b*) collection into a single device that directs the sample into 2 separate tubes, (*c*) collection using a single collection device subsequently subdivided into 2 specimens, and (*d*) serial collections performed using 2 separate devices within a defined period. Collection devices must be cleared by the Food and Drug Administration (FDA). Two different types of collection methods currently exist: a donor spits into a container or collection is performed with an absorbent pad device (i.e., cotton swab) (1). The FDA website contains a searchable database (2) to locate oral fluid collection devices cleared by the FDA.

In addition to permitting federal agencies to perform drug testing on oral fluids, the OFMG provides procedures for federal agencies to follow in the event an oral fluid cannot be collected. If the donor is unable to provide an oral fluid specimen, an alternate specimen type may be collected if specifically authorized by the federal agency and it meets the Mandatory Guidelines for Federal Workplace Drug Testing Programs. Such alternate testing will require approval by the employer, and possibly discussion with the MRO, before specimen (e.g., urine) collection (1).

The HHS Oral Fluid Drug Test Panel targets the same drug classes as urine testing (3), but the oral fluid cutoffs are lower because of lower analyte concentrations in oral fluid. A comparison of the oral fluid and urine cutoffs (indicated in parentheses) is shown in Table 1 (1, 3). Some differences in target analyte are owing to the higher prevalence of parent drug in oral fluid compared with urine. Of note, the marijuana target analyte in urine is the tetrahydrocannabinol (THC) metabolite δ-9-tetrahydrocannabinol-9-carboxylic acid (THCA) compared with THC in oral fluid, although both analytes are detectable in oral fluid. There is a discussion in the preamble of the HHS Guidelines regarding the relative value of THCA as a marker for THC that is informative (1).

The OFMG requires MROs to be trained on revisions to the guidelines before performing MRO duties for federal agency specimens. However, requalification will remain with the MRO certification entities, and references to continuing education unit requirements were removed from the final OFMG. HHS does not require validity testing on oral fluids, but validity testing is authorized upon request by the MRO. The OFMG provides examples of validity tests that the MRO may order, such

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Table 1.

Initial Test Analyte	Initial Test Cutoff, ng/mL[a]	Confirmatory Test Analyte	Confirmatory Test Cutoff Concentration, ng/mL
Marijuana (THC)[b] (urine THCA)[c]	4 (urine, 50)[d]	THC (urine THCA)	2 (urine, 15)
Cocaine/benzoylecgonine	15 (urine, 150)	Cocaine	8
		Benzoylecgonine	8 (urine, 100)
Codeine/morphine	30 (urine, 2000)	Codeine	15 (urine, 2000)
		Morphine	15 (urine, 2000)
Hydrocodone/hydromorphone	30 (urine, 300)	Hydrocodone	15 (urine, 100)
		Hydromorphone	15 (urine, 100)
Oxycodone/oxymorphone	30 (urine, 100)	Oxycodone	15 (urine, 100)
		Oxymorphone	15 (urine, 100)
6-Acetylmorphine	4 (urine, 10)[d]	6-Acetylmorphine	2 (urine, 10)
Phencyclidine	10 (urine, 25)	Phencyclidine	10 (urine, 25)
Amphetamine/methamphetamine	50 (urine, 500)	Amphetamine	25 (urine, 250)
		Methamphetamine	25 (urine, 250)
MDMA/MDA[c]	50 (urine, 500)	MDMA[c]	25 (urine, 250)
		MDA[c]	25 (urine, 250)

[a] For grouped analytes (i.e., 2 analytes that are in the same drug class and have the same initial test cutoff):
Immunoassay: The test must be calibrated with one analyte from the group identified as the target analyte. The cross-reactivity of the immunoassay to the other analyte(s) within the group must be 80%; if not, separate immunoassays must be used for the analytes within the group.
Alternate technology: Either one analyte or all analytes from the group must be used for calibration, depending on the technology. At least one analyte within the group must have a concentration equal to or greater than the initial test cutoff or, alternatively, the sum of the analytes present (i.e., equal to or greater than the laboratory's validated limit of quantification) must be equal to or greater than the initial test cutoff.
[b] An immunoassay must be calibrated with the target analyte, δ-9-tetrahydrocannabinol (THC).
[c] THCA, δ-9-tetrahydrocannabinol-9-carboxylic acid; MDMA, methylenedioxymethamphetamine; MDA, methylenedioxyamphetamine.
[d] Alternate technology (THC and 6-AM): The confirmatory test cutoff must be used for an alternate technology initial test that is specific for the target analyte (i.e., 2 ng/mL for THC, 2 ng/mL for 6-AM).

as for a biomarker (albumin or IgG) or for a specific adulterant (1).

Research Triangle Park Institute, the same contractor for HHS-approved urine drug testing, will continue to manage the National Laboratory Certification Program with the addition of oral fluid testing (4). A laboratory seeking HHS certification must take the following actions before testing federal agency specimens: (*a*) submit an application form approved by the Office of Management and Budget; (*b*) have its application accepted by HHS; (*c*) successfully complete the performance testing (PT) challenges in 3 consecutive sets of initial PT samples; (*d*) fulfill all requirements for an initial inspection; and (*e*) receive notification of certification from HHS. To maintain HHS certification, the laboratory must be inspected 3 months after certification and then every 6 months (1).

Full details on OFMG may be found at the Substance Abuse and Mental Health Services Administration (SAMHSA) website: https://www.samhsa.gov/workplace/resources.

Learning Objectives

After reading this article, the reader will be able to describe the changes to the Mandatory Guidelines for Federal Workplace Drug Testing Programs with the approval by the HSS for oral fluid specimen testing.

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Learning objectives vary by article, but in general, after completing *Clinical & Forensic Toxicology News*, the reader will be able to:

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- 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.
- Discuss relevant regulations, such as analytical performance requirements, or the legality of new drugs of abuse.
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- Describe the medical implications of drug abuse, toxicity associated with therapeutic agents, and exposure to other toxicants.

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