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Medication Assisted Treatment

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The Opioid Crisis

The genesis of the opioid epidemic in the United States can be traced to a shift in medical attitudes and practices related to the treatment of intractable chronic pain beginning in the mid-1980s. Treatment of pain with prescription opioids increased gradually through the early 1990s; however, the introduction of new opioid-based products and formulations such as the widely advertised OxyContin led to a surge in prescriptions and use of these drugs in pain management (1). The rapid increase in opioid use was predicated on the idea that these drugs had a low addiction potential when used to treat pain. What followed is an addiction crisis that continues today.

Between 1999 and 2018, almost 450,000 people in the United States died from overdoses involving opioids, either prescription or illicit. According to the Centers for Disease Control and Prevention (CDC), overdose deaths came in 3 distinct waves. The first, beginning in the late 1990s, correlated to increases in the use of prescription opiates and opioids for the management of chronic pain. As the addictive potential of prescription opioids became more widely recognized, access to these medications became more limited by changes in both federal and state statutes. In 2014 the Drug Enforcement Administration (DEA) moved hydrocodone combination products from Schedule III to Schedule II under the Controlled Substance Act (CSA), which eliminated prescription refills and increased restrictions on how prescriptions are dispensed (2). As a consequence, patients with burgeoning addictions began to use cheaper and more readily available illicit opioids. The second and third waves of overdose deaths beginning in 2010 and 2013 were largely due to overdoses involving heroin (2010) followed by synthetic opioids (2013), especially illicitly manufactured fentanyl (3). In 2017, the US Department of Health and Human Services (HHS) declared the opioid crisis a public health emergency. While the prescription opioid-related overdose deaths decreased by 13.5% between 2017 and 2018, death rates from synthetic opioids increased by 10% in the same time frame (4).

At present, approximately 2.1 million people in the United States are identified as having opioid use disorder (OUD), which is defined as a problematic pattern of opioid use leading to distress (5). In addition, there have been multiple reports of increases in drug overdoses related to decreased social contact, feelings of isolation, and stress resulting from the Coronavirus pandemic (6, 7). Early in the pandemic, the CDC recommended postponement of preventative medical care that could not be performed virtually, which contributed to a decreased availability of treatment and testing for drug and alcohol use disorders (8). Reduced access to treatment services may also play a role in the increased rate of overdoses.

Prevention and Treatment

Overdose prevention is comprised of multiple approaches including improving opioid prescribing practices as well as improved and expanded treatment options. Medication-assisted treatment (MAT) is an evidence-based treatment paradigm for substance use disorders that incorporates FDA-approved medications along with counseling and behavioral therapies into a "whole-patient" centered approach. Research has demonstrated that

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this combination treatment paradigm can help to sustain recovery (9). As a result of the documented efficacy and increasing adoption, MAT programs have become widely accepted in the United States and the number of providers is growing (10).

There are three primary drugs approved for use in MAT; methadone, buprenorphine, and naltrexone. Methadone is a long-acting opioid that has been used for decades in the treatment of opioid addiction. By law, this medication must be administered under supervision in a Substance Abuse and Mental Health Services Administration (SAMHSA)-certified opioid treatment program. Methadone can reduce withdrawal symptoms and drug craving; however, it is a strong opioid in its own right, and its use must be monitored carefully.

Approval of buprenorphine in 2002 greatly expanded OUD treatment options because it can be prescribed/dispensed in physician offices and other outpatient settings. Like methadone, buprenorphine is an opioid; however, it is a partial agonist that has a "ceiling effect" whereby drug effects increase with dose to a "ceiling" and then plateau without further increases regardless of dose (11). As a result, buprenorphine has a lower risk of dependence or misuse and fewer side effects compared to methadone. It may be administered alone (Subutex™) or in combination with the opioid antagonist naloxone (Suboxone™). The combination formulation is administered orally, by a sublingual tablet or a film applied to the inside of the mouth. This route of administration potentially minimizes diversion and/or misuse by taking advantage of differential oral absorbance efficiencies of the components. As naloxone is poorly absorbed via the oral route, the Suboxone formulation provides the benefit of buprenorphine with minimal contribution by naloxone. If administered by alternate routes, e.g., crushing followed by inhalation or injection, naloxone acts as an antagonist to the opioid effects.

Naltrexone is also an opioid antagonist approved for the treatment of substance use disorders (SUD), including alcohol use disorders (AUD). It is available in a pill form that is primarily used for AUD. An extended-release injection that improves patient compliance was approved for AUD in 2006 and OUD in 2010. Naltrexone can be prescribed by any licensed health care practitioner but is used more commonly in treatment programs based on abstinence, and it may also be used to prevent relapse in certain patients (12).

Traditional opioid treatment programs (OTP) in the United States that dispense opioid agonist medications for treatment purposes are certified by SAMHSA and must meet the requirements of 42 CFR 8.12 which sets forth Federal opioid treatment standards (13). Certified programs are required to provide medical, counseling, educational, and other assessment and treatment services in a structured manner, including a minimum of eight random drug screens per year for each patient in maintenance treatment. In many cases, those in outpatient treatment must visit the center daily, where the daily medication dose is administered under supervision. While this model has been in use for many years, the paradigm can be disruptive to a patient's daily routine. In addition, OTP centers may not be available in all geographic areas, limiting the accessibility of treatment.

The Drug Addiction Treatment Act of 2000 expanded the availability of MAT by permitting physicians with certain qualifications to prescribe and/or dispense Schedule III, IV, and V opioid medications for the treatment of OUD outside of traditional OTPs (14). Initially, the act limited the number of patients treated by a practitioner or practice group to 30 at a time, however that limit has since been raised to 100 based on the Comprehensive Addiction and Recovery Act of 2016 (15). These changes both expanded access to treatment and shifted the treatment paradigm to a model more similar to those used to manage other chronic illnesses. In a typical Suboxone program, the patient visits the physician's office weekly or monthly for treatment and receives a prescription for their medication. Though these programs are still required to include counseling and compliance monitoring in addition to prescribing OUD medications, the programs permit the integration of treatment into a more normalized lifestyle.

Laboratory Testing and MAT

Treatment professionals agree that monitoring drug use during treatment can be a powerful incentive for abstinence and can provide an early indication of a return to drug use. Like any other chronic disease, successful treatment for addiction requires ongoing evaluation and modification of the treatment plan as necessary (16).

SAMHSA-certified OTPs are required to provide random testing for drugs of abuse to monitor compliance with treatment regimens. The testing is used to monitor both the presence of non-prescribed drugs, which may be evidence of a relapse, as well as the absence of prescribed medications, which may be an indication of diversion. The frequency of testing is determined by the treatment professional based on patient risk assessments in the context of clinical appropriateness and the patient's stage of treatment (13). The Federal guidelines for OTPs suggest monitoring a

variety of drug classes, which may include opioids, benzodiazepines, cocaine, marijuana, amphetamines, barbiturates, and ethanol metabolites.

Laboratories providing testing for MAT programs offer multiple options ranging from discrete immunoassay panels, with or without confirmatory testing, to comprehensive drug screens using a definitive methodology. However, MAT programs are generally comprised of a different patient population with different testing needs than pain management patients, and such broader-based panels are generally not recommended (17). Test panels and testing frequency are designed to monitor compliance with the treatment regimen and prescribed drug or drugs, as well as potential diversion and/or relapse demonstrated by the presence of drugs of abuse. While urine remains the most commonly used sample matrix, the use of oral fluid is increasing due to ease of collection and minimized opportunities to substitute or adulterate a specimen.
Point of collection testing (POC) using a variety of lateral flow devices is also common, although, in isolation, these tests are not considered sufficient to satisfy the Federal requirements for drug testing (13).

For Suboxone programs, testing for buprenorphine, norbuprenorphine, and naloxone is a cornerstone component of the drug test panel. While naloxone has limited bioavailability when taken by mouth, urine from patients using Suboxone routinely shows measurable concentrations of buprenorphine, norbuprenorphine, and naloxone. The presence of the parent drug and its primary metabolite and the ratio or relationship to one another can also assist clinicians in the interpretation of test results (18–20). Donor urine containing only buprenorphine or buprenorphine and naloxone may be evidence of "pill dipping" or "scraping," a method that may be used by donors to adulterate a urine drug test specimen with a small fraction of unconsumed buprenorphine tablet or film to create the impression of compliance with the prescription medication. Testing protocols that rely on qualitative screening without confirmatory testing, including POC test devices, may be vulnerable to misinterpretation of test results in these cases.

In general, the correct interpretation of drug testing results is recognized as a key component for the successful incorporation of testing into patient care. Physicians involved in MAT programs must be aware of factors that affect test results, including patient-specific physiologic factors and metabolic pathways, as well as the details of laboratory testing. The importance of the provider's toxicology knowledge base is emphasized in several places, including the Federal OTP guidelines (13).

Summary

The OUD treatment landscape has changed dramatically over the past 20 years, reflecting the increase in prescription and illicit opioid use that began in earnest in the early 2000s. Treatment programs that use MAT, particularly with combination drugs like Suboxone that can be dispensed in advance, have become a widely accepted and successful approach to manage patients with OUD. Random drug testing is a cornerstone of these opioid treatment programs, as it can be used to confirm compliance and detect relapse. As the number of treatment programs continues to increase, urine and oral fluid drug testing are likely to remain a key offering for toxicology laboratories that serve MAT programs.

Learning Objectives

After reading this article, the reader will be able to:

- describe the opioid crisis and list factors that contributed to the emergence of the crisis
- define MAT and OTP and explain the services that these treatment programs offer
- summarize the role that laboratory testing plays in supporting the care of patients in MAT programs

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Iso-tonita-what? Isotonitazene, a Designer Opioid and an Emerging Threat

By Hunter Chamma and Sarah B. Riley, PhD

The illicit drug scene continues to evolve as drug distributors and clandestine chemists work to stay ahead of federal regulations and detection. So-called "designer" drugs make up a lot of this dynamic landscape (1). For several years, fentanyl analogs comprised the majority of designer drugs on the market, but these are now being eclipsed by other novel psychoactive substances, such as designer benzodiazepines and non-fentanyl-related designer opioids. Isotonitazene is one of the most recent designer opioids to emerge on the scene in the United States.

Isotonitazene, or N,N-diethyl-2-(2-(4-isopropoxybenzyl)-5-nitro-1H-benzimidazol-1-yl) ethan-1-amine, is a synthetic opioid of the benzimidazole class. Benzimidazole-derived opioid analgesics include etonitazene and clonitazene, which were developed in 1950 by CIBA Pharmaceuticals (now Novartis), but were never clinically approved or marketed for human use due to dangerous respiratory depression produced by the drugs (2-4). The benzimidazoles are structurally distinct from naturally occurring opiates, semi-synthetic opioids, and synthetic opioids such as fentanyl, fentalogues, and methadone. Etonitazene, clonitazene, and other benzimidazole derivatives are classified as Schedule I drugs by the Drug Enforcement Agency (5). With its powerful dependence potential, etonitazene has been used in drug addiction studies (6). Etonitazene has exhibited an affinity for the μ-opioid receptor 2500-fold higher than that of morphine, and 1000 times the potency of morphine in animal models (2). The pharmacological profile of isotonitazene is similar to that of its analog. *In vitro* studies have demonstrated μ-opioid receptor activation, recruitment of the regulatory protein β-arrestin-2, and an efficacy 180% that of hydromorphone (7, 8).

Isotonitazene was first discovered in March 2019 in Belgium (8). Other nations that have witnessed isotonitazene cases include Canada, the United Kingdom, Estonia, Germany, Latvia, and Sweden. Within the latter four nations, there were 24 cases reported between April 2019 and January 2020. The drug first emerged in the United States in April 2019 when 1.6 grams of the compound was confiscated by the United States Customs and Border Protection in California (5). After this case in California, the drug spread throughout the nation with a focus in the Midwest region; 85% of post-mortem cases involving isotonitazene from August 2019 to April 2020 were from the Midwest region (9).

Isotonitazene has been discovered in the form of powders, capsules, tablets, and liquid; by itself or cut with other substances. Powders have been described as brown, white, and yellow. In one instance, it was sold in powder form as etonitazene (5). It has been found most frequently in combination with designer benzodiazepines. From August

2019 to January 2020, isotonitazene was discovered compounded with etizolam (6 cases), flualprazolam (7 cases), fentanyl (6 cases), heroin (3 cases), tramadol (2 cases), and U-47700 (5 cases). In February 2020, 1900 isotonitazene tablets were confiscated in Halifax, Nova Scotia. These tablets were in the form of a white triangle with an "M" embossed on one side and an "8" embossed on the other side. There have not been any other cases of isotonitazene reported from Nova Scotia since then (4). While the distribution of isotonitazene is worldwide, the substance appears to come from outside of the United States and is sold online (10). Due to the increasing emergence of isotonitazene, the Drug Enforcement Agency temporarily characterized the substance as a Schedule I drug as of August 20, 2020 (11).

A recent study by the Center for Forensic Science Research and Education (CSFRE) reported the prevalence of isotonitazene in forensic casework submitted to NMS Labs between August 2019 and January 2020 (12). Authentic cases were determined as presumptively positive for isotonitazene by data-mining liquid chromatography-time of flight mass spectrometry data files collected during routine toxicological analysis of casework. The data-mining consisted of targeted data processing using the following input: name, formula ($C_{23}H_{30}N_4O_3$) converted to exact protonated mass, and a retention time of 5.656 minutes based on analysis of standard reference material. Cases with presumptive positive blood samples were submitted for confirmatory evaluation by liquid chromatography tandem mass spectrometry and quantitation by standard addition. In addition to postmortem blood, isotonitazene was also quantified in urine and vitreous fluid associated with each case (when available). Eighteen distinct cases were evaluated to include 18 blood samples, 6 urine samples, and 1 vitreous fluid sample. The average concentrations were determined as follows: blood, 2.2 ng/mL (0.4–9.5 ng/mL); urine, 2.4 ng/mL (0.6–4.0 ng/mL); and vitreous fluid 0.1 ng/mL.

Four metabolites of isotonitazene were also discovered in this evaluation of forensic casework. Based on structure, isotonitazene could potentially undergo N-dealkylation, O-dealkylation, both N-dealkylation and O-dealkylation, or nitro reduction. The resulting metabolites respective to the listed types of metabolism are N-desethyl-isotonitazene, O-desalkyl-isotonitazene, N-desethyl-O-desalkyl-isotonitazene, and 5-amino-isotonitazene. The CSFRE study found that the N- and O-dealkylation products were the primary urinary metabolites, and 5-amino-isotonitazene was the primary metabolite discovered in most of the blood samples.

A review of the case histories associated with these samples revealed trends typical of opioid overdose and opioid related deaths. History of opioid use, track marks, or other physical findings consistent with intravenous drug use, and autopsy findings consistent with opioid toxicity, such as pulmonary and cerebral edema, were noted in many of the 18 cases. While fentanyl, tramadol, and heroin were co-detected in some of the isotonitazene-positive cases, in half of the cases isotonitazene was the only opioid detected. Similar to reports of analysis of seized physical evidence, the most common co-detected drugs in these cases were designer benzodiazepines (flualprazolam, 7 cases; etizolam, 6 cases).

The St. Louis University Forensic Toxicology Laboratory, which provides toxicology services to the St. Louis, MO, metropolitan area, added isotonitazene to the general comprehensive screen applied to all casework on August 3, 2020. This screen is an information-dependent acquisition (IDA) method on a Sciex X500R QqTOF system equipped with an Exion liquid chromatography system. The limit of detection of isotonitazene by this method, determined by serial dilution of reference material in blank blood, is 0.12 ng/mL. Since initiating this screen, no cases of isotonitazene have been detected. The laboratory is currently undergoing re-analysis of all casework submitted from August 2019 through July 2020—including cases suspicious for opioid involvement where no opioids were detected—to determine whether any cases were missed prior to the development of this screen.

The emergence of isotonitazene on the drug scene is a threat because of its toxicity, its apparent use with other central nervous system (CNS) depressants, and difficulty in detection both for forensic chemists and toxicologists. Physically, it seems to be a chameleon appearing in a variety of colors and forms. In human biological specimens, it has thus far been found in low- to sub-nanomolar ranges, which requires sensitive instrumentation for detection. Being structurally distinct from other frequently identified opioids means that cross-reactivity with less specific methods (immunoassay, for example) is probably unlikely. For many laboratories, identification of isotonitazene may rely on astute recognition of possible involvement in a case–such as negative opioid results in a case with strong history for opioid involvement–and collaboration with a reference laboratory. Hopefully, the recent DEA scheduling will deter a significant increase in isotonitazene distribution and use. Regardless, toxicologists and clinical chemists should be aware of the potential for this drug, and ever vigilant for the next new threat.

Learning Objectives

After reading this article, the reader will be able to describe how isotonitazene differs from natural opiates, semi-synthetic opioids, fentanyl, fentalogues, and methadone. The reader will be able to list drugs commonly co-detected with isotonitazene in forensic casework.

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A Short Review of Nerve Agents

By Pradip Datta, PhD, DABCC

Nerve agents have been the topic of conversation in many news reports lately. Nerve agents are organophosphosporus compounds that affect the nervous system and produce a cholinergic syndrome. Exposure may occur occupationally, through organophosphate pesticides, or less commonly, as a result of bioterrorism. Synthetic nerve agents such as soman (GD), tabun (GA), sarin (GB), and VX have been used in bioterrorist attacks (Fig. 1). Recently, Novichok agents have been used for assassination attempts. Novichock is a group of nerve agents that are chemically very similar to VX, but differ by alkyl substituents on the nitrogen and oxygen atoms.

VX is one of the most potent known nerve agents where as little as 10 mg can kill a human within 10 minutes (1). Table 1 compares the lethality of 4 such agents. The lethal dose (LD50) is the amount of a toxic agent that is sufficient to kill 50 percent of a population of animals usually within a certain time. The lethal concentration (LCt50) is the concentration level (Ct) of an agent vapor that will be lethal to half of the population exposed to it. The median first noticeable effect concentration-time (MCt50) is the median first noticeable effect

Chemical Structures of Nerve Agents and Acetylcholine

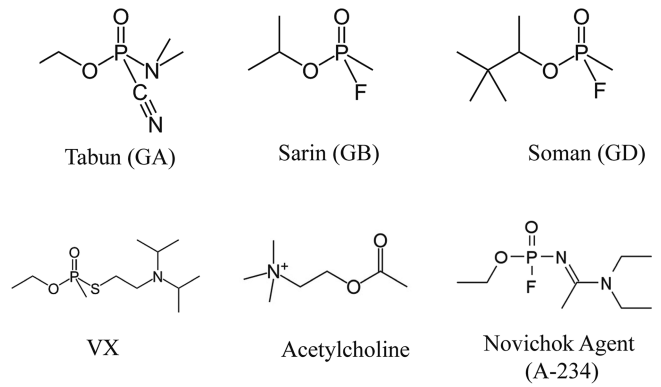

Fig. 1. Chemical structures of the nerve agents (vs acetylcholine).

Table 1. Key Properties of the nerve agents

Nerve agent	Tabun	Sarin	Soman	VX
LD50 (liquid) in a 70 kg man, mg	1000	1700	350	10
LCt50 (vapor), mg-min/m^3	400	100	50	10
MCt50 (vapor), mg-min/m^3	2–3	3	<1	0.04
Volatility (at 25°C), mg/m^3	610	22 000	3900	10.5
Aging $T_{1/2}$[a]	13.3–14 h	3–5 h	2–6 min	48 h

[a] h = hours; min = minutes.

concentration-time of miosis, or excessive shrinking of the pupil. The nerve agent VX is the least volatile but the most stable among these 4 nerve agents. VX is much more readily absorbed through dermal contact, making it primarily a contact hazard (2). Research has shown that VX can persist in soil from 2 to 6 days (2). Most of these nerve agents are organic phosphates which work by inhibiting acetylcholinesterase (AChE), an enzyme that catalyzes the breakdown of acetylcholine (ACh), a neurotransmitter.

AChE (EC 3.1.1.7) has a critical role in terminating nerve transmission by rapidly hydrolyzing ACh present in the synapse. AChE is expressed primarily at neuromuscular junctions in the peripheral nervous system (PNS) and cholinergic synapses in the central nervous system (CNS). It is also found in the lungs, spleen, the gray matter of the brain, and on the surface of red blood cells. Nerve agents directly inhibit AChE by binding to the active site at a critical serine residue, which prevents the ACh substrate from binding, resulting in ACh accumulation in the synapse and a cholinergic toxidrome. Once in the active site, the nerve agents can form a stable covalent bond with the serine by dealkylation of the phosphate group, resulting in "aging." The length of time it takes for aging to occur varies and can be as short as a few minutes for the nerve agent soman or take several days for the nerve agent VX.

Most nerve agents are volatile, colorless liquids. They can be absorbed through inhalation or skin contact. The symptoms of poisoning include chest tightening, difficulty in breathing, and potentially asphyxiation. Associated symptoms include vomiting and incontinence.

Detection of nerve agents can be accomplished indirectly by evaluating AChE activity. The most commonly used commercial assays measure the AChE activity via the Ellman chromogenic method, which is fast, accurate, and inexpensive. There are two different, but related, cholinesterase enzymes present in the blood; an AChE form that is on the surface of red blood cells (RBC-AChE) and serum butyrylcholinesterase (BChE, EC 3.1.1.8). Both can be used as surrogate markers for neuronal AChE inhibition confirming the initial clinical diagnosis of a nerve agent poisoning. Serum BChE, sometimes referred to as pseudocholinesterase, is often used to monitor acute poisonings because its activity declines and returns to normal more rapidly than RBC-AChE. Both AChE enzyme activities in the sample are measured by hydrolyzing a thiocholine ester substrate into an intermediate containing a free sulfhydryl. This then reacts with dithiobis-2-nitrobenzoic acid to form a yellow-colored product that is spectrophotometrically measured at 400 to 420 nm. The nerve agents decrease the acetylcholinesterase activity; a drop of more than 35% is considered severe poisoning.

Nerve agent poisoning can be treated with the antidotes atropine and pralidoxime chloride (2-PAM chloride). These antidotes have to be administered quickly; otherwise, the effect of the nerve agent cannot be reversed. These antidotes can also be used prophylactically. Benzodiazepines are currently the best treatment for seizures induced by severe nerve agent exposures and are administered intravenously or intramuscularly.

Since the discovery of nerve agents 100 years ago, the world bodies have prohibited their use in combat; however, there is concern about their use in clandestine wars and terrorist acts. Having a better understanding about nerve agents may help us better prepare for their potential use in the future.

Learning Objectives

After reading this article, the reader will be able to understand the nerve agents, their mechanism of action, laboratory analysis, and antidotes.

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Learning objectives vary by article, but in general, after completing *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.
- Discuss relevant regulations, such as analytical performance requirements, or the legality of new drugs of abuse.
- Explain the analytical and regulatory issues unique to specific applications, including postmortem toxicology, workplace drug testing, and drug screening.
- Describe the medical implications of drug abuse, toxicity associated with therapeutic agents, and exposure to other toxicants.

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