USING FORENSIC TOXICOLOGY SCREENING TO ENHANCE MEDICOLEGAL DEATH INVESTIGATIONS

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Abstract: The Milwaukee County Medical Examiner's Office performed an evidence-based review of natural deaths certified without autopsy or toxicology testing. 315 such cases from 2020 and 2021 were selected. This study reviewed the deaths to determine if the cause of death was drug-related versus natural disease. Blood samples were screened by liquid chromatography-quadrupole mass spectrometry with time-of-flight detection, a high-resolution mass spectrometry technique. The analytes detected were evaluated for contribution to the cause resulting in a change in manner of death from natural to accident or suicide. Confirmatory analysis was performed where appropriate and results were reported to the forensic pathologist for evaluation and amendment of the death certificate as appropriate. As a result of the screening and confirmatory work, 18 cases (5.7%) were identified where significant drugs were detected and thus the cause and manner of death were amended. One case was amended from natural to suicide after a conversation with the family about the toxicology findings, and the remaining cases were amended to a manner of accident. The confirmed substances that were deemed responsible for the deaths included both prescription medications and illicit drugs. These findings suggest that appropriate toxicology screening will assist with determination of cause and manner of death, even in cases that may have not been traditionally examined.

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Chapter I Introduction

Medicolegal death investigation is an area of forensic science that carries a unique responsibility. Scientists in this field are tasked with investigating deaths that are sudden, unexpected, or unattended and certifying them with proper cause and manner of death. The death investigation system varies by location. Some jurisdictions have a coroner system, and some have a medical examiner system. In general, coroners are elected, and medical examiners are appointed. Coroner systems will differ by jurisdiction. In some jurisdictions, the coroner is an elected official who lives in the jurisdiction and is at least 18 years of age. In some jurisdictions the coroner is part of the sheriff's department, while in other jurisdictions there is a medical examiner who oversees the death investigations in the jurisdiction. Medical examiners can have a varied level of education to support their position. Some are forensic pathologists, and some are not. Regardless of who is the administrator of death investigations in a jurisdiction, laws in the United States require that a medicolegal autopsy must be performed by a forensic pathologist. A forensic pathologist is an individual who has a medical degree, completed a residency in pathology, a fellowship in forensic pathology, and has been board certified in anatomic, forensic, and clinical pathology or anatomic and forensic pathology. This means that if a jurisdiction is overseen by an individual who does not have that level of credentialing,

they must seek a partnership with a locality that has that level of credentialing to administer an autopsy. The United States has a well-documented shortage of forensic pathologists [1-8]. Currently, there are fewer than 50% of the appropriate number of forensic pathologists for the workload that exists to date[9]. As the number of cases reported to an office continues to increase, the demand for individuals in this profession will continue to increase.

Death investigation is an area of forensic science that is multifaceted in the array of experts that contribute to the case data that is ultimately evaluated by a forensic pathologist or coroner prior to certification of cause and manner. There is an investigation of the scene and circumstances performed by a medicolegal death investigator and/or the police, the autopsy performed by a forensic pathologist, and other investigations such as toxicology or other ancillary tests performed at a forensic or clinical laboratory. All these resources can be utilized in the investigation of one single death.

Statement of the Problem

Jurisdictions throughout the United States are experiencing an increase in many causes of death, and drug-related deaths make up the greatest area of increases[10]. The opioid epidemic has been ongoing for years, and many areas have declared specific drugs, such as fentanyl, to be a public health crisis. Drug related deaths are a burden on offices across the country. The Centers for Disease Controls and Prevention (CDC) reported over 3.3 million deaths in 2020[11] and over 3.4 million in 2021[11]. The CDC reports that there were 91,799 drug related deaths in 2020[12]. Data from 2021 was not available. In comparison, Wisconsin reported 1,515 drug related deaths in 2020 and 1765 in 2021[13].

During those same years, Milwaukee County reported record numbers of drug-related deaths; 544 in 2020 and 644 in 2021. This equates to Milwaukee County reporting 36% of the State's drug-related deaths.

In response to the increasing number of deaths due to a wide-array of causes, including drug-related deaths, offices must make policy decisions to manage their case load. One way to mitigate case load is by limiting the number of full forensic autopsies. One way of reducing the number of autopsies is by identifying decedents that can be examined externally with or without collecting biological specimens for toxicological analysis. Another option to reduce the autopsy load is by releasing decedents to a funeral home without performing an extensive body exam and certifying the death based on a review of the associated medical records. For the purposes of this report, these cases will be referred to as body-release (BR) cases. The investigation in BR cases might consist of a scene investigation, records and information obtained from law enforcement and fire department partners, and medical records obtained from the patient's physician. Interviews with next-of-kin are relied upon heavily for information related to the patient's social and medical history. Additionally, samples of blood and or vitreous fluid can be obtained should the need for toxicology analysis arise over the course of the investigation. There is little standardization in this area of death investigations and even variance between investigators in the questions asked of family during an interview.

Deaths associated with a drug-related cause are increasing at a rate that is difficult for offices to keep pace with, especially given the shortage of practicing forensic pathologists. Agencies that are accredited by the National Association of Medical Examiners (NAME) must also take into consideration the standard that limits the

maximum number of autopsies a pathologist can perform annually. The current standard limits the number of cases per pathologist to 250 per year with a maximum of 325 cases per pathologist per year[14]. Policy choices to limit or eliminate testing in certain cases creates a risk that the cause of death may be inaccurately certified. In particular, a drug-related cause may be missed without toxicological analysis. This lack of testing could result in an inaccurate cause and manner of death and an underrepresentation of the number of drug-related deaths in a jurisdiction. There is little standardization[14] in this area of medicolegal death investigation, and as a result there is a variety of policy and practice across offices.

Purpose of the Study

This study provides evidence-based support for addressing policies that dictate the types of cases that should require a full toxicology screen associated with the death investigation even if a full forensic autopsy is not performed. The null hypothesis was that no body release deaths were improperly certified by cause or manner of death.

Significance of the Study

These results of this evidence-based study show that when toxicology was not utilized as part of the death investigation, the cause of death was mis-categorized. These results highlight the value that toxicology analysis can add to a death investigation for evidence that cannot obtained any other way.

Chapter II Review of Literature

Sparse literature exists that speaks to studies such as this one. Much literature exists that highlights the importance of a quality death investigation system and the limitations that currently exist in various medical examiner and coroner offices in the United States. The pandemic associated with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which causes coronavirus disease 2019 (COVID-19) made clear the need for accurate vital statistics[16]. These statistics are regularly leveraged for policy and practice decisions that affect the public at large. Civil registration and vital statistic systems are the best source for data related to all deaths as they should provide timely and accurate mortality data. If this data is not complete and accurate, then the relevance of the burden of non-natural deaths is minimized, and policy makers do not have the data they need to initiate preventive efforts[15]. Hanzlick[16] in 2014 reported, "over the past 82 years, needs for the death investigation system have been described repeatedly. Although progress has been made in meeting those needs and improving death investigation systems, much work remains to be done." In another article by Hanzlick[17], offices in the United States were surveyed to find perceived strengths and limitations. This survey afforded offices the opportunity to share their thoughts. It was noted in the report that the survey was

focused on support for a state medical examiner system. Where support for a statewide system did not exist, there was not a single respondent that could speak for the whole state and its needs or limitations. Many states could identify an array of strengths in their death investigation systems. They cited observations such as the local nature of their death investigations, strengths of the coroner systems, leadership of forensic pathologists in the investigations, and some even cited reasonable funding. On the flip side the respondents also cited perceived limitations in their death investigation systems. Some limitations cited include use of coroners, inadequate or limited training, lack of funding, limits in the investigation scope, and some reported low rates for autopsy which impacts quality.

A study performed by Nashelsky et.al[18] in 2003 evaluated the accuracy of the cause of death in cases where an autopsy was not performed. The authors retrospectively reviewed cases that had been autopsied and were complete and certified to identify cases that were natural deaths. The cases were then blinded to the autopsy findings and the remaining case information was assessed to determine a cause of death. The presumed cause of death was wrong in 28% of the cases and a nonnatural manner of death was present in 3% of the cases. The conclusion of this study was that even experienced forensic pathologists may generate erroneous death certificates if the case is not subject to autopsy. In the study 4 cases or 1.5% of their study population had a drug intoxication cause of death. The study was designed to focus on cases with an apparent natural manner of death and yet there were eight nonnatural deaths in the study. The outcomes of that study and this study impact public health and safety in a similar way. Both studies highlight the need for "(1) comprehensive accuracy of vital statistics and (2)

identification of the true prevalence of underdiagnosed causes of death for resource allocation in public health."[18]

There is a lot of focus on the utility of the autopsy and a presumption that you could miss detection of a natural cause of death, especially in a younger person, if an autopsy is not performed. However, you could miss a drug-related death if toxicology analysis is not performed.

The screening protocol prior to implementation of the liquid chromatography-quadrupole mass spectrometry with a time-of-flight detector (LC-QToF) utilized enzyme linked immunosorbent assay (ELISA). The kits utilized were a product from Immunalysis Corporation. The panel targeted 10 classes/individual compounds benzodiazepines, opiates, oxycodone, methadone, fentanyl, buprenorphine, cocaine metabolite, cannabinoids, amphetamine, and methamphetamine. This enzyme immunoassay (EIA) technology leveraged an antibody-antigen reaction to identify the presence of a class of compounds. EIA is generally considered a cost-effective way to do screening. EIA can be easily automated which is appealing especially to a high throughput laboratory. The sample preparation is simple and the interpretation essentially clear. For all these reasons, EIA is widely used in toxicology laboratories both clinical and forensic. However, sensitivity is a limitation with this technique and there is limited reactivity for various analytes that are within the classes being analyzed. For instance, the benzodiazepine assay targeted oxazepam for optimal reactivity. Other benzodiazepine compounds, particularly low dose benzodiazepines, do not cross-react well and therefore limit the sensitivity and selectivity for these analytes in the immunoassay. This is the situation for all kits that target a class of compounds as opposed to an individual analyte

and the metabolite(s). A kit that targets a particular class (i.e., benzodiazepines) or an analyte (i.e., fentanyl) is not designed to detect the variety of analogs or designer compounds that would emerge. This then requires that labs either verify and validate the level of cross-reactivity where the vendor had not or rely on an alternative way to screen for those analytes.

As this limitation was becoming more burdensome, the toxicology lab at the Milwaukee County Medical Examiner's office decided to purchase a liquid chromatograph time of flight mass spectrometer system (LC-QToF) to be able to specifically identify the analytes at the time of the screen and provide more accurate and timely identifications with a greater scope and better sensitivity. The justification for the change in technology was supported by improvement to the process by comparison of time, cost, and changing drug trends. The time associated with preparing samples for the screen by the traditional EIA was approximately 30 minutes. Analysis time by the automated liquid handler was about 3 hours. The interpretation of the results was about 30 minutes. The time for the sample preparation for the LC-QToF is about 2 hours, followed by approximately 30 minutes of acquisition time by the instrument, and data analysis time (which can vary based on the complexity of the sample results). The cost of EIA testing is about $1 per well. With the panel utilized at the time that total cost was $10 per sample plus costs to analyze quality control samples within each batch. The traditional acidic/basic/neutral (ABN) screen by GC-MS required 1-2 mL of sample as opposed to the 250-500 µL of sample for the LC-QToF. Furthermore, the changes in drug trends fully favor the utility of the LC-QToF. The other technologies do not have the ability to keep pace with the need for more sensitive and selective data acquisition.

There appears to be a shift in the forensic toxicology field from EIA to LC-QToF. Labs are evaluating this paradigm shift and recognizing the improvements in sensitivity and selectivity by changing to a high-resolution technology.[18-26] By changing to screening by LC-QToF, the laboratory expanded the screening capabilities and improved sensitivity and selectivity. The addition of new analytes of interest can be easily incorporated into the screen allowing the laboratory to keep better pace with the changes in trends. Additionally, this technology allows the laboratory to perform retrospective data analysis if an emerging substance becomes known. The lab can retrospectively evaluate a data file from previously acquire samples and look for newly trending analytes. The data for the samples in this study was acquired via this technology.

The array of analytes detected in the cases in this study where the death certificate was changed, primarily focuses around fentanyl and cocaine. These two substances were very frequently identified in casework in Milwaukee County at that time.

Cocaine is a highly addictive, schedule II stimulant that is generally found in two forms; cocaine base and cocaine salt. The base form is consumed via smoking and the salt form is consumed through varied routes of administration, injection, insufflation, transdermal, and absorbed through a mucous membrane (intranasal, sublingual, vaginal, rectal). The desired effects are euphoria and alertness, but toxic or fatal outcomes result from cardiotoxic and ischemic events. Cocaine is one of many things that can cause cardiovascular disease; therefore, investigating the nature of the cardiovascular disease in a death investigation could substantiate the need for toxicology testing. Dolinak[27] describes the pathophysiology of cardiotoxic effects of cocaine. The heart is stressed when cocaine is used; heart rate increases and blood pressure increases. Cocaine can

cause arrhythmias including sinus tachycardia, sinus bradycardia, supraventricular tachycardia, accelerated idioventricular rhythm, ventricular tachycardia, ventricular fibrillation, torsades de pointes, bundle branch block, compete heart block and asystole. Karch[28] discusses various studies that have been done to evaluate the effect of cocaine on diseased and non-diseased vessels. Karch also discusses the types of changes that can be observed in the vasculature of individuals who use cocaine and why individuals may experience sudden cardiac death. Some patients experience sudden cardiac death and Karch advises that the death investigation should consider the different mechanisms as the concentration of cocaine does not seem to be a related factor. The mechanism responsible seems to be that of a lethal arrhythmia, which is a fatal rhythm disturbance that occurs when the ventricular impulses are disturbed. Karch describes how cocaine can cause "distinct morphologic and physiologic changes in the heart." Cardiomegaly, or enlarged heart, will increase the distance that the impulses have to travel. In addition to morphology changes, cocaine is a also local anesthetic and can cause conduction disruption due to the repolarization from being a sodium channel blocker. While a sudden cardiac death can be certified as a natural manner of death, the presence of cocaine in the decedent's system changes that to a manner of accident.[29]

Fentanyl is synthetic opioid pain reliever that is listed as a Schedule II narcotic. While there are other receptor interactions, it primarily activates the Mu opioid receptor. This receptor is responsible for producing symptoms from euphoria and anesthesia to dose dependent respiratory depression. It is this respiratory depression that makes fentanyl so dangerous. When administered in a controlled environment like a hospital where the airway can be protected, it is very safe to use. When used illicitly, especially in solitude,

the respiratory depression can be toxic, if not fatal. The therapeutic concentration for fentanyl is generally very low, single digit nanogram per milliliter low. That therapeutic concentration can increase with tolerance.

Fentanyl is very easily synthesized and has seemingly taken the place of heroin as the most frequent opioid consumed. Frequency of heroin-related deaths have steadily declined in Milwaukee County in the past five years. The fentanyl used does not appear to be coming from a licit source in the United States. Pharmacies are not being burglarized for the drug like was happening for oxycodone years ago. Instead, illicit fentanyl has been coming into the United States via various routes most of which seem to have roots that lead back to China and Mexico as the two top producers[30]. A variety of other fentanyl analogs have made their presence known for a short time, and then seem to disappear. This trend is not uncommon among designer drugs and novel psychoactive substances. A variety of other substances have been identified in cases that contain fentanyl and the common question is whether or not those substances were co-occurring in the material that was consumed. Toxicology can not answer that question. Once the substances appear in the body, that is what is identified. It is unknown through toxicology testing if the individual consumed 1 substance or 2 or many to arrive at the toxicology results they have.

Chapter III Methods

In this research, 315 cases from 2020 and 2021 from the Milwaukee County Medical Examiner's Office were selected where neither an autopsy nor toxicology analysis had been performed (BR cases), the manner of death was certified as natural, and where blood was collected. This study aimed to determine if the cause of any of the deaths was from a drug-related cause instead of natural disease. This study was granted IRB exemption.

A case can be classified as a body release (BR) based on the following criteria. Generally, the decedent is at least 55 years of age and has a significant medical history that could support the death without the need for an autopsy. The case also needs to be one in which there is no physician or accredited practitioner who has attended or treated the decedent within 30 days preceding the death to sign the death certificate or the attending physician refuses to do so. Consequently, the case would become the jurisdiction of the medical examiner's office.

The laboratory information management system (LIMS) in use at the Milwaukee County Medical Examiner's Office was queried for cases from 2020 and 2021 that were designated as a body release in which the cause of death was natural, and blood was available for testing. The blood samples were screened by liquid chromatography-quadrupole mass spectrometry with a time-of-flight detector (LC-QToF).

The LC-QToF is a Waters Xevo G2-XS equipped with an Acuity I-Class Binary solvent manager, and an Acuity I-Class Sample manager with a flow through needle. The instrumental conditions are provided below, with the gradient method for positive and negative mode provided in Table 1 and Table 2, respectively. The system was operated with Unifi software version 1.9. The purchased Unifi library has over 1500 analytes and most were added to the screening method. The system was originally validated for over 100 drugs that are commonly identified in the laboratory and included in the Unifi library to be used as a screening method for use in identifying analytes that could contribute to the cause of death. The library is continuously updated with emerging analytes. This screening method was compared to the current practice at the time, which was utilization of enzyme immune assay and gas chromatography-mass spectrometry.

Liquid Chromatograph Conditions:

Column: Acquity HSS C18 2.1x150 1.8um
Column Temperature: 30°C
A1/B1: 5mM Ammonium Formate + 0.1% Formic acid/acetonitrile + 0.1% formic acid
A2/B2: Water + 0.001% Formic acid/acetonitrile + 0.001% formic acid
Injection Volume: 2.0 uL
Flow rate: 0.4 mL/minute

Table 1. Gradient LC Parameters for Positive Mode

Time (min)	Rate (mL/min)	%A	%B
0	0.4	87	13
0.5	0.4	87	13
10	0.4	50	50
10.75	0.4	5	95
12.25	0.4	5	95
12.5	0.4	87	13
15	0.4	87	13

Table 2. Gradient LC Parameters for Negative Mode

Time (min)	Rate (mL/min)	%A	%B
0	0.4	87	13
0.5	0.4	87	13
10	0.4	50	50
4.5	0.4	5	95
5.45	0.4	5	95
5.5	0.4	87	13
7.5	0.4	87	13

Mass spectrometer parameters:

Capillary Voltage: 1.0 kV
Source Temperature: 150℃
Desolvation temperature: 400℃
Gas flow: 800 mL/minute

LCMS ToF Filter Criteria

<u>High Confidence (Detected)</u>

1. Target Match tolerance = 5 PPM

2. Fragment(s) found >1

3. Fragment (F v E) Found vs. Expected = ≥ 50%

4. Generate predicted fragments from structure. Fragment match tolerance = ± 2.0 mDa

5. Ion ratio tolerance = 10%

6. Absolute retention time tolerance = 0.3 min

7. Response ≥ 2000 counts

8. Detector counts ≥ 5000

Moderate Confidence (Indicated)

1. Expected fragment count > 0. (ie…there has to be fragments in the library)

2. The analyte must be identified, based upon criteria from the Identified filter

3. Target Match tolerance = 10 PPM

4. Fragment Match tolerance = ± 5.0 mDa

5. Absolute retention time tolerance = 0.5 min

6. Response ≥ 1000 counts

7. Detector counts ≥ 2000 counts

Low Confidence (Inconclusive)

1. Expected fragment count > 0

2. The analyte must be identified

3. Target match tolerance = 10 PPM

4. Fragment match tolerance = ± 5.0 mDa

5. Absolute retention time tolerance = 0.5 min

6. Response = no minimum threshold required

7. Detector counts = no minimum threshold required

** All three confidence levels must display adequate extracted ion chromatography and resolution, which includes minimal background interference and acceptable signal to noise ratios. This is a subjective measure that is performed at the bench level by a qualified forensic chemist.

The sample preparation was deliberately chosen to leave the sample as raw as possible so as to not limit analytes from being detected. A sample preparation procedure was obtained by United Chemical Technology (UCT) which utilized Refine™ Ultra-Filtration

1mL solid-phase extraction (SPE) cartridges, part number RFNSPE1. The procedure utilized 250 to 500 µL of blood or urine. Internal standard was added to each sample to achieve a concentration of 20ng/mL of morphine-D3, fentanyl-D5, MDMA-D5, alprazolam-D5, benzoylecgonine-D3 and 100ng/mL of carboxy-THC-D3 and hexobarbital and 50ng/mL of amphetamine-D8. The protein was precipitated from each sample by vortexing after the addition of 1mL of cold acetonitrile. The samples were then centrifuged for 5 minutes. This supernatant was loaded onto the Refine™ SPE column and extracted on a UCT positive pressure manifold with a pressure less than 5psi of regulated flow. The eluate was collected and dried at 35°C for greater than 50 minutes. The sample was then reconstituted with 300µL of initial conditions mobile phase, [87% mobile phase A (5mM ammonium formate + 0.1% formic acid/acetonitrile + 0.1% formic acid) and 13% mobile phase B (water + 0.001% formic acid/acetonitrile + 0.001% formic acid)] and transferred to an autosampler vial equipped with an insert and capped. The sample was then ready for injection on the instrument.

The testing provided a targeted screen with a scope of approximately 800 compounds. The analytes detected were evaluated for potential substantial contribution to the cause of death which would also result in a change in the manner of death from natural to either accident or suicide. Confirmatory analysis was performed for analytes that could presumably contribute to the cause of death, and the results were reported to the forensic pathologist for evaluation with the case. Where the cause of death was determined to be from a drug-related cause, the death certificate was amended.

Chapter IV Method Validation

Blood samples were screened by LC-QToF. The LC-QToF was validated according to ANSI/ASB Standard 036 Standard Practices for Method Validation in Forensic Toxicology. In accordance with that standard, the sample preparation and instrument parameters were validated for use in a forensic toxicology laboratory. Results of the validation were summarized in Table 3.

Method validation included assessment of limit of detection, precision, carryover, and method comparison. For the limit of detection/precision study, analytes were grouped (25 or less) and calibration solutions prepared at a range of concentrations from 1ng/mL to 100ng/mL. A blank matrix was fortified with the appropriate concentration and analyzed in singlet over five (5) days. The limit of detection was determined with identification of analytes qualitatively on 4 of 5 days. Results of the limit of detection study were summarized in Table 4. For the carryover study, validated analytes were assessed at a minimum concentration of 1000 ng/mL. These were injected in triplicate and followed by a blank to assess for carryover. No carryover was identified in this experiment. The flowthrough needle is designed to greatly diminish the opportunity for carryover in the system by continuously cleansing throughout the run. While no formal study was performed for interference the analysis has identified a few analytes

where interference or specificity have created challenges. Ephedrine/pseudoephedrine, quinine/quinidine, positional isomers, and chiral compounds are some examples. When these compounds are detected in the screen, they are reported in a way that clearly speaks the limitation, for example, pseudoephedrine/ephedrine. The recovery of the internal standards was evaluated for precision. For the method comparison, the results from this screen were compared to the results obtained from EIA and GCMS screening. Method comparison compared 300+ samples to in-house EIA, GCMS, QToF screen data. 100% concordance with EIA and GCMS plus additional compounds identified and select compounds confirmed by LCMSMS or sent to a reference laboratory.

Table 3 Summary of Validation

Parameter:	Acceptance Criteria	Result
Bias	N/A	N/A
Calibration Model	N/A	N/A
Carryover	Validated analytes will be assessed at a minimum concentration of 1000 ng/mL. These will be injected in triplicate and followed by a blank to assess for carryover based on retention time, fragmentation, detector count, and response.	No carryover identified.
Interference Studies	N/A	Some interferences identified (Pseudoephedrine, Ephedrine; quinine/quinidine; chiral compounds)
Limit of Detection	Target: 1 ng/mL (Director assigned); qualitative	See Table; LoD Compilation
Precision	Qualitative	Analytes were identified at the LOD on a minimum of four out of five days.
Sample Stability	All analytes qualitatively identified on inter-day analysis.	Most analytes qualitatively were identified on inter-day analysis. Extracted stability: Two analytes with low response were not identified on day 2 Processed stability: One analyte was not identified on day 1 but was on day 3; a separate analyte with low response on day 1 was not identified on day 3.
Method Comparison		Compared 300+ samples to in-house EIA, GCMS, QToF screen data. 100% concordance with EIA and GCMS plus additional compounds identified and select compounds confirmed by LCMSMS or sent to NMS.

Table 4 Limit of Detection Summary

	LOD Concentration			
Analyte	1 ng/mL	5 ng/mL	10 ng/mL	50 ng/mL
10-OH Carbazepine				X
11-OH THC		X		
4-ANPP	X			
4-Methoxybutyrylfentanyl	X			
6-Acetylmorphine	X			
7-Aminoclonazepam	X			
Acetylfentanyl	X			
Acrylfentanyl	X			
Alprazolam	X			
Amitriptyline	X			
Amobarbital*			X	
Amphetamine				X
Benzoylecgonine	X			
Benxodioxole fentanyl	X			
Buprenorphine	X			
Butalbital				X
Butyryl Fentanyl	X			
Carisoprodal*				X
Chlordiazepoxide	X			
Chlorpheniramine	X			
Cis-3-methyl Fentanyl	X			
Citalopram	X			
Clonazepam		X		
Clonazepam			X	
Cocaethylene	X			
Cocaine	X			
Codeine	X			
Crotonyl Fentanyl	X			
Cyclobenzaprine	X			
Cyclohexyl fentanyl	X			
Cyclopentyl Fentanyl	X			
Cyclopropyl Fentanyl	X			
Delorazepam				X
Desalkylflurazepam		X		

Desipramine		X		
Dextromethorphan		X		
Diazepam	X			
Diclazepam		X		
Diphenhydramine			X	
Doxepin	X			
Doxylamine	X			
Duloxetine		X		
EDDP (Methadone metabolite)	X			
Ephedrine	X			
Estazolam				X
Etizolam		X		
Fentanyl	X			
Fluoroisobutyrylfentanyl	X			
Flualprazolam		X		
Flubromazelam			X	
Flubromazepam				X
Fluoxetine	X			
Fluvoxamine		X		
Furanyl Fentanyl	X			
Gabapentin		X		
Hydrocodone	X			
Hydromorphone	X			
Imipramine	X			
Isotonitazene	X			
Ketamine	X			
Lamotrigine	X			
Loperamide	X			
Lorazepam		X		
Lysergic acid diethamide	X			
Methylenedioxyamphetamine			X	
Methylenedioxymethamphetamine	X			
Methadone	X			
Methamphetamine		X		
Methoxyacetyl Fentanyl	X			
Methylphenidate	X			
Metonitazene	X			
Midazolam	X			
Mirtazapine	X			

Mitragynine			X	
Morphine	X			
Naloxone		X		
N-desmethyl doxepin	X			
Norbuprenorphine	X			
Norfentanyl	X			
Norfluoxetine			X	
Norsertraline				X
Nortriptyline		X		
ODM-Venlafaxine	X			
Ortho-methyl-furanyl fentanyl	X			
Oxazepam		X		
Oxazepam				X
Oxcarbazepine		X		
Oxycodone	X			
Oxymorphone		X		
Para-Fluorobutyl Fentanyl	X			
Para-methyl acetyl fentanyl	X			
Paroxetine	X			
Phencyclidine			X	
Phenazepam				X
Phenobarbital*				X
Phentermine				X
Phenyl fentanyl	X			
Phenytoin*				X
Phenylpropanolamine				X
Pregabalin				X
Propoxyphene			X	
Pseudoephedrine	X			
Quetiapine	X			
Sertraline		X		
Tapentadol	X			
Temazepam	X			
Tetrahydrofuran Fentanyl	X			
Tramadol	X			
Trans-3-methyl Fentanyl	X			
Trazodone	X			
Valeryl Fentanyl	X			
Venlafaxine	X			

Xylazine	X			
Zolpidem	X			

*Negative ionization analytes

The method comparison data showed good concordance. Over 300 case samples were ultimately evaluated in the validation to show how results compared between the EIA screen, the GC-MS screen (if/where performed) and the LC-QToF screen. The screening results were supported by the necessary confirmatory work by GCMS or LCMSMS where dictated by the case investigation. Two of these cases comparisons are highlighted here. A blood sample was analyzed by the 10 panel EIA with no drugs or drug classes indicated. The GCMS screen indicated the presence of bupropion, caffeine, gabapentin, bupropion metabolites, acetaminophen, cotinine, and ibuprofen. The LC-QToF screen indicated the presence of each of those analytes except cotinine and ibuprofen, but additionally identified loperamide, N-didesmethylloperamide, metoprolol and trazodone. Confirmatory work by LCMSMS identified the presence of loperamide at 100ng/mL and the metabolite was confirmed. Had the LC-QToF screen not been performed, this acute loperamide toxicity may not have been detected. In another case, the EIA indicated the presence of benzodiazepines, cannabinoids, amphetamine, and methamphetamine type compounds. The GCMS screening indicated the presence of alprazolam, caffeine, cotinine, methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA). The LC-QToF screening indicated the presence of all those analytes and additionally benzoylecgonine, lysergic acid diethylamide (LSD) naloxone, ibuprofen, and carboxy-THC glucuronide. In this case, the LSD could have been missed if not for the LC-QToF screening.

Chapter V: Findings

The dataset consisted of a total of 315 cases across two years (2020 and 2021) and was analyzed from both a quantitative and qualitative perspective. In 2020, 143 cases were identified, and 172 cases were associated with 2021. Among the 315 cases, 18 were identified as positive (5.7%). Eight positive cases were from 2020 and 10 positive cases were from 2021. In relation to the positive cases 13 were male and 5 were female. The males had an average age of 59 years with a median of 60 years and a range of 43-70 years. The females had an average age of 61 years with a median of 62 and a range of 52-72 years. Nine (50%) were black, 7 (39%) were white, and 2 (11%) were Asian. Although half of the cases were black individuals, that is the race with the greatest increase in rate of death in the jurisdiction. Demographic information is provided in Table 5.

Table 5 Decedent Descriptive Statistics

Descriptive statistics regarding age both aggregated and disaggregated by sex among cases where the cause of death was changed.

	Mean	**Median**	**SD**	**Min, Max**	**SEM**	**95% CI**	**N**
Male	59	60	8.8	43, 70	2.4	53, 64	13
Females	61	62	7.6	52, 72	3.4	51, 70	5
All Subjects	59	60	8.3	43, 72	1.9	55, 63	18

Note: SD = standard deviation; Min, Max = minimum and maximum age; SEM = standard error of the mean; 95% CI = 95% confidence interval of the mean; N = sample size

Quantitative Analyses

The data were first analyzed to determine if any differences were present among the medicolegal death investigators that investigated each case. In total, 13 medicolegal death investigators were associated with the 315 cases. Twelve out of the 13 medicolegal death investigators handled the 18 cases that were identified as positive (Table 6).

Table 6 Positive Cases by Investigator

The total number of positive cases assigned to each medicolegal death investigator (N = 13)

MDI	1	2	3	4	5	6	7	8	9	10	11	12	13
Cases	1	3	1	3	2	1	2	1	1	1	1	1	0

Note: MDI = Medicolegal death investigator

Some investigators had multiple cases over the two years. Two cases in this sample set that were reported to the office at nearly the same time on the same day and were investigated by two different investigators. Two investigators had three cases, two had two cases and the rest were single case per investigator. A chi-square goodness of fit test was conducted among the 12 investigators that handled the positive cases to determine if the observed distribution of positive cases they handled statistically significantly differed from the expected distribution. The chi-square goodness of fit test was not statistically significant, χ^2 (11) = 4.66, p = .95. In other words, the observed frequencies were distributed as expected. However, it should be noted, given the small sample size, the expected frequencies were <5, ergo, caution should be taken when interpreting the results.

The day of the week and the time of the day each positive case was reported was also examined in addition to the number of total cases the medicolegal death investigator examined during that time (Table 7). Three of the positive cases were reported on Sunday, 2 on Monday, 3 on Tuesday, 4 on Wednesday, 2 on Thursday, 3 on Friday, and 1 on Saturday. The hours of the day were broken into 6 four-hour blocks. Block 1 was from 0000 hours to 0359 hours, block 2 from 0400-0759 hours, block 3 0800-1159 hours, block 4 1200-1559 hours, block 5 1600-1959 hours, and block 6 2000-2359 hours. Two cases were reported in block 1, 2 cases in block 2, 3 cases in block 3, 6 cases in block 4, 4 cases in block 5, and 1 case in block 6. This is consistent with the patterns of time during the day that cases are reported to the office. During 2020 and 2021, approximately 63% of cases were reported between 0800-2000 hours.

Table 7 Calendar Effects on Reported Positive Cases

The day of the week and the time of the day each positive case was reported in addition to the number of total cases the medicolegal death investigator examined during that time.

Case	Age	Sex	Ethnicity	MDI	DoW	Time Block	Total Cases
A	53	Male	White	1	Sunday	4	3
B	60	Male	White	2	Wednesday	4	6
C	54	Male	White	3	Monday	4	5
D	55	Male	Black	4	Tuesday	3	2
E	70	Male	White	2	Wednesday	6	4
F	43	Male	Asian	5	Sunday	5	3
G	44	Male	Asian	6	Tuesday	2	3
H	68	Male	Black	7	Tuesday	2	3
I	60	Male	Black	8	Friday	3	3
J	63	Male	White	9	Wednesday	2	3
K	62	Female	Black	2	Friday	5	4
L	72	Female	Black	5	Friday	5	3
M	56	Female	Black	12	Sunday	4	4
N	62	Female	Black	10	Monday	5	3
O	67	Male	Black	4	Saturday	3	4
P	52	Female	Black	4	Thursday	4	3
Q	59	Male	White	11	Wednesday	4	7
R	68	Male	White	7	Thursday	2	2

Note: MDI = Medicolegal death investigator; DoW = Day of week
Time Blocks:
Block 1 = 0000-0359 hours
Block 2 = 0400-0759 hours
Block 3 = 0800-1159 hours
Block 4 = 1200-1559 hours
Block 5 = 1600-1959 hours
Block 6 = 2000-2359 hours
Total Cases = the total number of cases reported during that time block

The total number of cases that fell within each day by time block when a positive case was reported is displayed in Table 8. In reference to the time blocks, the greatest frequency of cases was reported during block 4. Specifically, 28 (43.08%) of the total cases (N = 65) were investigated at this time. This block has an overlap of staffing from first and second shift. As previously stated, this time block also contained the highest

frequency of positive cases (n = 6). The two cases that were mentioned earlier that were reported at nearly the same time in block 2 that were investigated by two different investigators only had one other case reported during that time block. The results from a chi-square goodness of fit test indicated the observed frequencies were not distributed as expected, $\chi^2(4) = 25.08$, $p < .001$. In reference to the day of the week, the greatest frequency of cases was reported on Wednesday. Specifically, 20 (30.77%) of the total cases (N = 65) were investigated on this day. This day contained 4 (22.22%) of the identified positive cases (n = 18). Again, results from a chi-square goodness of fit test indicated the observed frequencies were not distributed as expected, $\chi^2(6) = 17.82$, $p = .007$

Table 8 Cases by Day and Time

Contingency table displaying the total number of cases per day and time block

		Time Block					
		Block 2	Block 3	Block 4	Block 5	Block 6	**Total**
Day	Sunday	0	0	7	3	0	10
	Monday	0	0	5	3	0	8
	Tuesday	6	2	0	0	0	8
	Wednesday	3	0	13	0	4	20
	Thursday	2	0	3	0	0	5
	Friday	0	3	0	7	0	10
	Saturday	0	4	0	0	0	4
Total		11	9	28	13	4	65

Note: Time Blocks:
Block 1 = 0000-0359 hours
Block 2 = 0400-0759 hours
Block 3 = 0800-1159 hours
Block 4 = 1200-1559 hours
Block 5 = 1600-1959 hours
Block 6 = 2000-2359 hours

As a result of the screening and confirmatory work, 18 cases were identified in which significant drugs of abuse were detected and thus the cause and manner of death were amended. One case was changed from natural to suicide after a conversation with the family about the toxicology findings. The other 17 cases were changed to a manner of accident and the scope of drugs responsible for the deaths included prescription and illicit. The original cause of death in these cases were primarily cardiovascular, n = 10 (55.56%), 2 (11.11%) were complications of diabetes mellitus, and 6 (33.33%) were due to chronic obstructive pulmonary disease. After comprehensive testing, the cause was amended to acute cocaine intoxication, n = 7 (38.89%), and mixed drug intoxications, n = 10 (55.56%) that include opioids (fentanyl, heroin, oxycodone, methadone, acetyl fentanyl, fluorofentanyl) and each were ruled a manner of accident. One case (5.56%) was due to an acute venlafaxine intoxication and was ruled a suicide (Table 9). Fentanyl was present in 44% of the cases.

Table 9 Original and Amended Cause of Death

The original cause of death and amended cause of death among all positive cases (N = 18)

Case	Original Cause of Death	Amended Cause of Death
A	Atherosclerotic coronary vascular disease	Acute mixed drug (methadone, fentanyl, acetylfentanyl, morphine) intoxication
B	Complications of diabetes mellitus	Acute heroin intoxication
C	Hypertensive cardiovascular disease	Acute venlafaxine intoxication
D	Hypertensive cardiovascular disease	Acute cocaine intoxication
E	Chronic obstructive pulmonary disease	Acute fentanyl intoxication
F	Complications of diabetes mellitus	Acute fentanyl intoxication
G	Acute myocardial infarct	Acute mixed drug (fentanyl, heroin) intoxication
H	Hypertensive and atherosclerotic cardiovascular disease	Acute mixed drug (fentanyl, heroin, methadone) intoxication
I	Chronic obstructive pulmonary disease	Acute cocaine intoxication
J	Atherosclerotic coronary vascular disease	Acute mixed drug (morphine, oxycodone) intoxication
K	Chronic congestive heart failure	Acute cocaine intoxication
L	Chronic obstructive pulmonary disease	Acute cocaine intoxication
M	Atherosclerotic coronary and peripheral vascular disease	Acute mixed drug (cocaine, fentanyl) intoxication
N	Chronic obstructive pulmonary disease	Acute cocaine intoxication
O	Hypertensive and atherosclerotic cardiovascular disease	Acute cocaine intoxication
P	Atherosclerotic coronary vascular disease	Acute cocaine intoxication
Q	Chronic obstructive pulmonary disease	Acute mixed drug (fentanyl, meta/para fluorofentanyl) intoxication
R	Chronic obstructive pulmonary disease	Acute mixed drug (fentanyl, meta/para fluorofentanyl) intoxication

Qualitative Analyses

Among the 18 positive cases, qualitative data were analyzed to identify any themes that could point to a history of drug use. Further, the identification of themes may

provide recommendations to medicolegal investigators in identifying a drug related death at the time of the scene response or when conducting interviews with next of kin. Fourteen (78%) of the positive cases had no mention of pain or drug use while 4 (12%) had a mention of pain or drug use. One of those cases made mention of old medications on scene, another case had mention of four prescriptions, another mentioned a basket of medications, but all appeared to be in order (used as prescribed). Another was reported to a hospital so there is no record of drugs from the scene but a mention of alcohol abuse.

Six cases in which a scene investigation occurred made some mention of drugs on scene. Two cases mentioned marijuana and some prescriptions which appeared in order or taken less than prescribed. One case mentioned that prescription medications appeared in order. Another case noted that there was alcohol and medications but no illicit drugs. One case mentioned a vape device and other medications being in order. The last mention was of medications on scene but no illicit drugs.

Family opposition was a consideration for rationale as to why a case was classified as a body release. Only one case mentioned family opposition. The family was opposed to autopsy and even the body being brought to the office for external examination, citing a religious objection.

The presence of a foam cone from the nose or mouth can be present in cases of drowning or congestive heart failure. A foam cone can also be consistent with, but not diagnostic, of a drug-related death. Case narratives were reviewed for mention of a foam cone. Two of the cases noted a foam cone. One was a mention of a small amount of foam coming from the decedent's nose and blood from the nose and mouth. No trauma

was noted. The other case made mention of a foam cone from a decedent who had a history of congestive heart failure and a remote (7 years prior) history of drug use.

The relationship of the person who first encountered the decedent and made the report was investigated for themes. In 13 or 72% of the cases the person to discover the decedent was a friend or family member. Some were friends or relatives that lived with the decedent, and some were cases where the decedent lived alone, and family were the first to encounter the decedent during a welfare check. Two cases were self-report emergencies. In one case it was unknown who found the decedent and another case was found by a bystander. The deaths that were first encountered by a friend or family member pose the concern that the scene could be cleaned up prior to reporting the death to law enforcement. Scene cleanup could be done to protect the family or friends from the stigma of substance use. In scenes where a medicolegal death investigator did not report to the scene, the details from the scene were reported by first responders who responded to the scene. First responders are not trained in the same way as a medicolegal death investigator. Where evidence of substance use that is in plain sight should be recognized and documented by either party, jurisdiction is not the same for searching concealed areas. Investigations of deaths are not the primary duty of a first responder who may not be a detective nor a paramedic from the fire department.

One of these cases made mention of some old medications on scene. Another case had mention of some prescriptions on scene for oxycodone, tramadol, Chantix® and prednisone. Because no scene response was performed, no pill count was available. The oxycodone on scene was noted to have been filled on 5/22/20 and a review of the electronic prescription drug monitoring program (ePDMP) listed a prescription for

oxycodone for a 30-day supply (90 pills). Fentanyl, norfentanyl, caffeine, and cotinine were identified in this decedent's blood.

The array of toxicological findings was evaluated. Some identified substances were determined to contribute the death where others were not listed on the death certificate. Not all substances identified from the screen were confirmed. All the analytes named on the death certificate were confirmed. Most cases where the death certificate was amended had drugs that could have been obtained illicitly. One case where the death certificate was amended had a prescription drug that was determined to be the sole contributor to the death. Many cases had additional drugs identified but it was opined that those substances did not contribute to the cause of death. One case had one other substance, 3 cases had 2 other substances, 3 cases had 4 other substances, 2 cases had 5 other substances, 1 case had 6 other substances, 7 cases had 7 other substances, 8 cases had 8 other substances, 1 case had 10 other substances, 1 case had 11 other substances, and 1 case had 15 other substances. Figure 1 displays the frequency of other analyctes found across all cases. Table 10 contains the specific substances per case. Stability of analytes is always a concern in toxicology. Stability can vary greatly from one analyte to another and storage can be a great concern. All samples in this study were stored at -20C from the time they were received at the lab until the time they were tested. Stability of all analytes isn't known, so it is important to recognize that some analytes may have been missed merely due to stability and the time duration between collection and analysis. All samples were tested within 12 months of collection with most analyzed within 30 days.

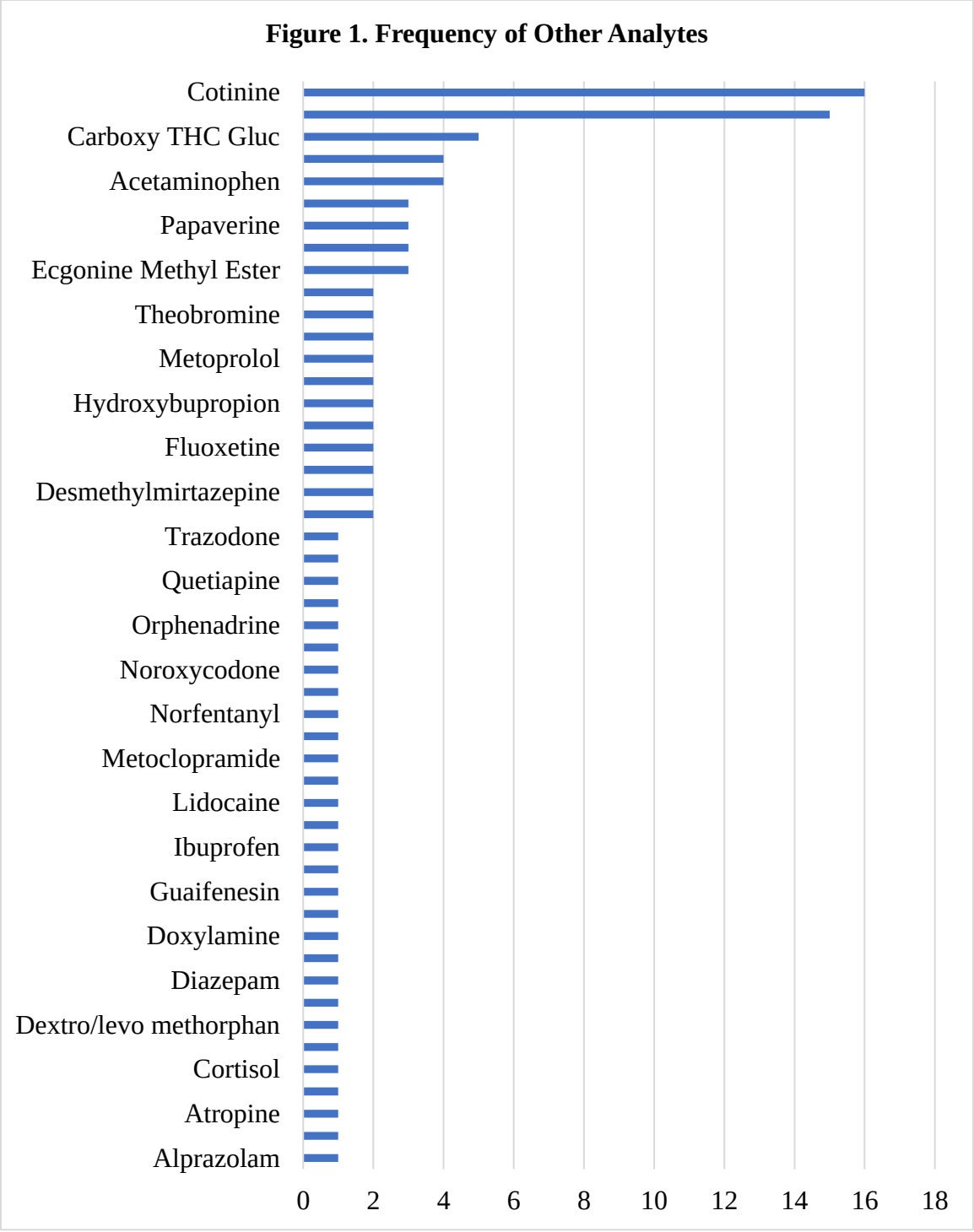

Figure 1 Frequency of Other Analytes.

Table 10 Other Analytes Detected by Case

Other substances detected per case

Case	Other Substances
A	Caffeine, cotinine, ibuprofen, quinine/quinidine
B	Nicotine, lidocaine, caffeine, cotinine, papaverine, orphenadrine
C	Propranolol, doxylamine
D	Caffeine, carboxy, THC-glucuronide, cotinine, EME, labetalol
E	Caffeine, cotinine
F	Caffeine, cotinine, fluoxetine, hydroxybupropion, metoclopramide, norfluoxetine, gabapentin
G	Acetaminophen, papaverine, caffeine, cotinine
H	Diphenhydramine, mirtazepne, caffeine, cotinine, nicotine, papaverine, quinine/quinidine
I	Caffeine, cotinine
J	Acetaminophen, carboxy THC-glucuronide, caffeine, cotinine, dextro/levo methorphan, dextrorphan/levorphanol, nicotine, noroxycodone
K	Acetaminophen, furosemide, caffeine, cotinine, EME
L	Alprazolam, bupropion, caffeine, cotinine, hydroxybupropion
M	Carboxy THC glucuronide
N	Diclofenac, furosemide, caffeine, cotinine
O	Carboxy THC glucuronide, cotinine, desmethylmirtazepine, levamisole, mirtazapine, phenylephrine, trazodone
P	Atropine, caffeine, cotinine, EME, fluoxetine, levamisole, nicotine, norquetiapine, phenylephrine, quetiapine, theobromine
Q	Azithromycin, caffeine, cotinine, carboxy THC glucuronide, diphenhydramine, theophylline, diazepam, nordiazepam, temazepam, quinine/quinidine
R	Acetaminophen, atorvastatin, azithromycin, caffeine, cotinine, cortisol, desmethyldiltiazem, desmethylmirtazepine, guaifenesin, hydroxymetoprolol, losartan, metoprolol, mirtazapine, theophylline, norfentanyl

Note: EME = ecgonine methyl ester

Qualitative Descriptions for Each Case

Case A: A 53-year-old male who was found dead on his couch by family. No one interviewed had any medical history to share. He was reportedly tired after a night of snow removal. He resided his two cousins. There was no mention of any substance use. There was no scene response by a medicolegal death investigator. The original cause of

death was atherosclerotic coronary vascular disease with a manner of natural. The amended cause of death was acute mixed drug (methadone, fentanyl, acetyl fentanyl, morphine) intoxication with a manner of accident.

Case B: A 60-year-old male as found dead in bed after being sick from an infection on his neck. He had a history of diabetes, a leg amputation 3 years prior, and an unhealing would on his neck. He was found by a neighbor. He lived in a rooming house. There was no scene response by a medicolegal death investigator. His original cause of death was complications of diabetes with a manner of natural. His amended cause of death was an acute heroin intoxication and a manner of accident.

Case C: A 54-year-old male was found dead in bed in a room he rented from a friend. This was a rooming house situation. He had a history of hypertension, alcoholism and a self-declared decline in health. There was no scene response by a medicolegal death investigator. His original cause of death was hypertensive cardiovascular disease and a manner of natural. The amended cause of death is an acute venlafaxine intoxication. Upon notification to the family of the amended cause of death, the daughter advised that she was certain that her father's death was a suicide as he had been calling suicide hotlines and expressing suicidal ideations. Because of that confession, the amended manner of death was suicide.

Case D: A 55-year-old male with no medical history was found dead, nude on his living room floor. He was found by his daughter and ex-wife. The scene investigation from the medicolegal death investigator reported the presence of a small amount of dried foam and blood draining from the nose and mouth. There was marijuana present on scene. The

original cause of death was hypertensive cardiovascular disease with a manner of natural. The amended cause of death was an acute cocaine intoxication with a manner of accident.

Case E: This was the eldest male in the cohort. A 70-year-old male with a history of emphysema and prescription drug abuse was found dead in his kitchen. It was unknown who called 911. There was no scene response from a medicolegal death investigator. First responders reported the presence of prednisone, oxycodone, tramadol, and Chantix. His sister reported he had a 40-year history of abusing drugs. His drug of choice was Percocet, but he would use anything. His original cause of death was chronic obstructive pulmonary disease with a manner of natural. His amended cause of death was an acute fentanyl intoxication and a manner of accident.

Case F: This is the youngest male in the cohort. A 43-year-old male with a history of diabetes and alcoholism. He was found by family who entered the home via a ladder. The scene response from the medicolegal death investigator documented prescriptions that appeared in order. There were probable veinous puncture sites, but the decedent had recently been hospitalized for alcohol intoxication and gastritis. Family was opposed to autopsy, so specimens were drawn on scene in case toxicology were to be needed in the investigation. No toxicology testing was ordered. The original cause of death was complications of diabetes with a manner of natural. The amended cause of death was an acute fentanyl intoxication with a manner of accident.

Case G: a 44-year-old male presented to the emergency department with an elevated troponin level. No hospital drug screen was performed. Due to the hospital presentation, no scene response was performed by a medicolegal death investigator. The original

cause of death was an acute myocardial infarct with a manner of natural. The amended cause of death was acute mixed drug (fentanyl, heroin) intoxication with a manner of accident.

Case H: A 68-year-old male called 911 for shortness of breath. He was dead upon arrival of emergency medical personnel. No scene response was performed by a medicolegal death investigator. The police officer reported the observation of an opioid abuse program card with an appointment for a previous date. There was no noted evidence of drugs or paraphernalia. His son reported that his father uses marijuana and has a history of cocaine use 30 years prior. The original cause of death was hypertensive and atherosclerotic cardiovascular disease with a manner of natural. The amended cause of death is acute mixed drug (fentanyl, heroin, methadone) intoxication with a manner of accident.

Case I: a 60-year-old make who was found dead on the floor by his couch. He had his walker nearby and an oxygen tank with the nasal canula in place and the oxygen running. He was found by family and the building manager upon a welfare check. His original cause of death was chronic obstructive pulmonary disease, and the manner was accident. The amended cause of death was an acute cocaine intoxication with a manner of accident.

Case J: A 63-year-old male who had a witnessed arrest at his residence. He lives with his girlfriend and her son. The son had put him to bed about 0200 hours and found him sitting upright complaining of in ability to breathe around 0730 hours. There were no drugs in the electronic prescription drug monitoring program (ePDMP). No medicolegal death investigator responded to the scene. The original cause of death was

atherosclerotic coronary vascular disease, and the manner was natural. The amended cause of death was an acute mixed drug (morphine and oxycodone) with a manner of accident. The significance of the lack of drugs in the ePDMP is that both morphine and oxycodone are prescription drugs that would be documented in that record. Morphine can also present in cases as a metabolite of heroin. A vitreous sample was analyzed additionally in this case for the presence of 6-monoacetylmorphine which was not detected. Had 6-monoacetylmorphine been present, the source of the morphine could have been at least in part from heroin.

Case K: A 62-year-old female was found dead in her home. The medicolegal death investigator that went to the scene noted the presence of a foam cone. The daughter of the decedent said her mom had congestive heart failure. The daughter also reported that her mom hadn't used drugs in 7 years. Also noted on scene was a lighter and vape pen. The original cause of death was chronic congestive heart failure, and the manner was natural. The amended cause of death was an acute cocaine intoxication with a manner of accident.

Case L: This was our oldest female in the cohort. A 71-year-old female had a witnessed collapse in front of family who attempted cardiopulmonary resuscitation. There was no scene response by a medicolegal death investigator. She had a history of chronic obstructive pulmonary disease and hypertension. The original cause of death was chronic obstructive pulmonary disease and a manner of natural. The amended cause of death waws an acute cocaine intoxication with a manner of accident.

Case M: A 65-year-old female found down in her home. She was found by her grandson. No scene investigation was performed by a medicolegal death investigator. The decedent's son had reportedly died a couple months prior. His death had a manner of natural. This was followed up on to see if her death could have been a suicide associated with grief. Nothing was identified to substantiate that. Her original cause of death was atherosclerotic coronary and peripheral vascular disease with a manner of natural. Her amended cause of death was an acute mixed drug (cocaine, fentanyl) intoxication with a manner of accident.

Case N: A 62-year-old female was found down at home by family. She had a history of chronic obstructive pulmonary disease. A medicolegal death investigator responded to the scene and reported that the decedent was found clutching an albuterol inhaler. Her original cause of death was chronic obstructive pulmonary disease, and the manner was natural. The amended cause of death was an acute cocaine intoxication, and the manner is accident.

Case O: This case is the youngest female in the cohort. A 52-year-old female was found unresponsive in her bathroom by her 13-year-old daughter. The decedent was last known well 30 minutes prior. When she didn't emerge from the bathroom, the daughter entered and found her unresponsive. There was no scene response by a medicolegal death investigator. Her original cause of death was atherosclerotic coronary vascular disease, and the manner was natural. The amended cause of death was an acute cocaine intoxication with a manner of accident.

Case P: A 68-year-old male was found dead in a running vehicle. There were black and mild cigars, a breathing apparatus, and inhalers found in the vehicle. The decedent had a medical bracelet on his wrist from a hospital visit the week prior. The original cause of death was chronic obstructive pulmonary disease, and the manner was natural. The amended cause of death was an acute mixed drug (fentanyl, meta/para fluorofentanyl) intoxication and a manner of accident.

Case Q: A 59-year-old male with a history of chronic obstructive pulmonary disease was found down in his bedroom. There was no scene investigation performed by a medicolegal death investigator. The decedent had recently been seen for covid positivity. The original cause of death was chronic obstructive pulmonary disease, and the manner was natural. The amended cause of death was an acute mixed drug (fentanyl, meta/para fluorofentanyl) intoxication with a manner of accident.

Case R: A 62-year-old male was found in his residence. He was nude on a couch. There was a leafy green substance on scene. He was found by an apartment manager. His original cause of death was hypertensive and atherosclerotic cardiovascular disease, and the manner was natural. The amended cause of death was an acute cocaine intoxication with a manner of accident.

Conclusion

It is noted that this study was performed during a world-wide pandemic. Milwaukee County, Wisconsin was no different in the impact the pandemic caused. Because of the pandemic, the office chose to make several changes in the way death investigations were conducted. Early in the pandemic, these changes were to reduce

potential exposures to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which causes coronavirus disease 2019 (COVID-19). An exposure to someone with COVID-19 at that time resulted in a 14-day period of quarantine. The impact that repeated or wide-spread quarantine periods could have on an office staff was profound. Therefore, in an effort to reduce exposures, scene response was limited to cases of suspected homicide or where staffing allowed. During every shift there were investigators working in the office, from home, and some who were off of work completely. Regardless of whether an employee was investigating a death from home or in the office, the option to have an investigator respond to a scene was still available. Of the 18 cases from this study where the death certificate was changed, eleven (11) or 61% of the investigations did not have a scene response.

Chapter VI Implementation

Implementation of additional testing like this takes courage and the support of both the toxicology laboratory and the department of pathology. Some strong emotions can come into play when quality assurance procedures are put into place and current policy is questioned or challenged. That being said, it is important to understand the risk of a mis-certification. As this project has progressed, the impacts on the decedent's family of amending the death certificate have been discussed. One consideration is their emotional distress, perhaps learning that a death was a suicide and not an accident. It is not known how the amended death certificate might affect things like death or life insurance payouts that have been made or are pending. Consideration of outward effects and consequences serves as a source of bias on the one providing the opinion, which is something that forensic science diligently works to prevent. In this instance, the forensic pathologists that are responsible for identifying the manner and cause of death need to work in a space that is free from influences of bias.

With an eye on an opportunity for quality improvement, an office can decide to evaluate cases like this where there is presumably an explainable cause of death, by doing toxicologic analysis to ensure there is sufficient investigation to accompany conclusions. The evidence to support the presence of a drug or toxin is only obtained with analytical toxicology work.

Implementation of a comprehensive level of toxicology testing in an office that has an in-house toxicology laboratory can have a minimal impact on workload. Samples are routinely batched in toxicologic analysis, and the case volume should be able to be handled in step with other case analysis in the laboratory. For offices that do not have an in-house laboratory, the cost to send these samples to a reference lab can vary based on scope of testing. The time associated with the testing is a consideration for offices as well. The turnaround time for analysis is an important consideration and is also a standard that must be met for offices accredited by NAME. Ninety percent of cases need to be completed in 60 days and another standard requires ninety percent to be completed in 30 days[11]. Ideally the toxicology analysis would be performed within a short time frame so that the certification of death would not be delayed. Quality in the investigation and accuracy in the certification should be at the forefront of the goals of implementation.

Chapter VII Conclusions

Each family was called to inform them of the amendment to the death certificate. The responses from family varied from surprise to indifference. Families that were indifferent could be interpreted perhaps as having some prior knowledge of the substance use. The unanswered question is why that information was not shared with the investigator at the time of the investigation of the death and how can that be gleaned more efficiently in the interview process. An office deciding to include toxicological analysis to any but particularly these death investigations does not add burden on the already understaffed pathology department.

This study was limited in that the entire study set was from one office, the Milwaukee County Medical Examiner's Office. This is a large metropolitan office, consequently there are differences in amenities and policy and practice. Most notably is the existence of an in-house toxicology laboratory. Only two years' worth of cases were evaluated. Of that two years' worth of cases, only a subset of them had blood available for testing. Only cases where the original cause of death had a manner of natural were evaluated. Had all BR cases (with blood available for testing; regardless of manner of death) been included the number of cases would have been 690. If blood had been routinely collected on all BR cases, there were 2,361 cases that could have potentially been affected in just those 2 years.

Recommendations for future study would include an analysis of cases from a medical examiner or coroner office where there is no forensic pathologist on staff and where decisions about when and where to do an autopsy are limited financially. It would be beneficial to look at a sample set from an office that is not a large metropolitan office. It would also be interesting to look at the policies and practices as it pertains to categorization of body release cases (however named).

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