This is to certify that the
thesis entitled

DISTRIBUTION OF METHADONE AND EDDP
IN POSTMORTEM TOXICOLOGY CASES

presented by

Jessica Amanda Jennings

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Forensic Science

Major Professor's Signature

4/22/05

Date

DISTRIBUTION OF METHADONE AND EDDP IN POSTMORTEM TOXICOLOGY CASES

By

Jessica Amanda Jennings

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE IN FORENSIC SCIENCE

School of Criminal Justice

2005

ABSTRACT

DISTRIBUTION OF METHADONE AND EDDP
IN POSTMORTEM TOXICOLOGY CASES

By

Jessica Amanda Jennings

Methadone, a legal synthetic opioid, has been used for the treatment of heroin and morphine addiction for over 40 years now in addition to being used as an analgesic. This drug is less addictive than its opioid counterparts, but it is still abused. Consequently, methadone accounts for a large portion of drug deaths each year. At the State of Delaware Office of the Chief Medical Examiner, 100 methadone-related deaths from September 6, 2001 through March 1, 2005 were analyzed to determine the distribution of methadone and its main metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in these cases. The cases were divided into six groups based on their causes and manners of deaths. Methadone toxicity is difficult to interpret due to overlapping concentrations in therapeutic and lethal concentrations. Several factors contribute to this overlap including tolerance and individual variability in metabolism. Postmortem redistribution also complicates interpretation. The main specimens that were analyzed in this study included vitreous humor, peripheral blood (femoral), heart blood, brain, gastric contents, and liver. Average concentrations of methadone and EDDP were determined for each specimen in each group. The group in which death was due to methadone toxicity alone had the highest concentration of methadone for all specimen types with the exception of liver. In addition, ratios of parent drug to metabolite and ratios of vitreous, brain, and liver to blood were calculated. These ratios provided valuable information in some instances, but methadone deaths still remain a challenging category of deaths.

This thesis is dedicated to my Mom and Dad with love and gratitude.

ACKNOWLEDGEMENTS

I would like to first thank Dr. Rebecca Jufer Phipps for all of her guidance and generosity throughout this research project. She is the one who proposed the project and also the one who served as my primary advisor. Even though she no longer supervises or works with me, she continued to help me, which I greatly appreciate. Dr. Jufer has provided a wealth of knowledge and assistance over the past year. She has taught me basically everything I know about forensic toxicology. Additionally, I would like to acknowledge Dr. Jay Siegel who also served as an advisor and committee member. Dr. Siegel is one of the main reasons I decided to go to Michigan State—due to his outstanding reputation in forensic science. He has been an inspirational person and role model ever since I met him. Also serving on my committee is Dr. Sheila Maxwell. I would like to thank her for her time and concern as well.

I owe a huge thank-you to the staff of the State of Delaware Office of the Chief Medical Examiner for allowing me to partake in this project using their instruments and supplies. I would like to especially thank John Marino and Rick Pretzler for their special efforts on my behalf. Thank you also to my family and friends for all of their love and support. My parents and sister have always encouraged me and believed in me. I cannot express my gratitude enough to my parents—for both their emotional and financial support. I could not have accomplished this goal without them. I owe a warm special thank-you to my Mom for her countless hours of proofreading. Finally, I would like to thank the love of my life—my fiancé Jeffrey. He and our kitten Gracie have kept me smiling throughout this entire endeavor.

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KEY TO SYMBOLS AND ABBREVIATIONS

Symbol or Abbreviation	Meaning
°C	degrees Celsius
>	greater than
/	per
%	percent
±	plus or minus
(±)	racemic
®	Registered trademark
µL	microliter
µm	micrometer
A	accident
AA	African American
ACS	American Chemical Society
alpha-1-AGP	alpha-1-acid glycoprotein
Ante	antemortem
ASCAD	arteriosclerotic coronary artery disease
ASCVD	arteriosclerotic cardiovascular disease
Bld	blood
C	Caucasian
C/P	central-to-peripheral
CNS	central nervous system
COD	cause of death
COHb	carboxyhemoglobin
(cont'd).	continued
COPD	chronic obstructive pulmonary disease
CYP	cytochrome P450
d_3	deuterated
DI	deionized/distilled
dL	deciliter
e.g.	example given
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EMDP	2-ethyl-5-methyl-3,3-diphenylpyrroline
F	female
g	gram
GC	gas chromatography

KEY TO SYMBOLS AND ABBREVIATIONS (cont'd).

Symbol or Abbreviation	Meaning
GC (in tables)	gastric contents
H	Hispanic
HB	heart blood
Hep C	Hepatitis C
Hg	mercury
HIV	human immunodeficiency virus
Hosp	hospital
HPLC	high-performance liquid chromatography
HTCVD	hypertensive cardiovascular disease
HTN	hypertension
kg	kilogram
L	liter
m	meter
M	male
MDN	methadone
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mM	millimolar
MMT	methadone maintenance treatment
MOD	manner of death
Mol. Wt.	molecular weight
MS	mass spectrometry
n	number of positive cases
N	natural
NA	not applicable
ND	none detected
ng	nanogram
NPD	nitrogen-phosphorus detection
OCME	Office of the Chief Medical Examiner
P	pending
PB(Fem)	peripheral blood (femoral)
PB(Sub)	peripheral blood (subclavian)

KEY TO SYMBOLS AND ABBREVIATIONS (cont'd).

Symbol or Abbreviation	Meaning
pH	potential of hydrogen
pKa	ionization constant
PM/AM	postmortem-to-antemortem
R	Rectis (Latin for right)
RPM	revolutions per minute
S	Sinister (Latin for left)
S (in Table A1)	suicide
SD	standard deviation
SIM	selected ion monitoring
SML	serum methadone level
SPE	solid-phase extraction
U	undetermined
UCR	urinary cortisol ratio
UCT, Inc.	United Chemical Technologies, Incorporated
VH	vitreous humor

INTRODUCTION

Aims of this Project

In Delaware, there have been 100 methadone-related deaths between September 6, 2001 and March 1, 2005. According to the records of the State of Delaware Office of the Chief Medical Examiner (OCME), there were six deaths in the final four months of 2001. Twenty-three deaths involving methadone toxicity occurred in 2002, 37 in 2003, and 28 in 2004. In the first three months of 2005, six deaths were associated with methadone.

Much research has been devoted to methadone-related deaths, but most of the published articles focus on establishing a lethal methadone concentration range. However, due to the overlapping concentrations in deaths attributable to methadone toxicity and those unrelated to it, this is practically impossible. Confounding factors such as tolerance and postmortem redistribution partially account for this overlap. There has been little research aimed at evaluating the distribution of parent drug to metabolite in such cases. One aim of this research project was to determine ratios of methadone to its main metabolite in Delaware's methadone cases over the past three and a half years to see if these would help in classifying such deaths. Since methadone is widely distributed in tissues such as the liver and kidney, it is possible that these specimens (that are typically not studied) could provide valuable information. In addition to assessing ratios, another goal was to determine methadone and metabolite concentrations in other matrices to determine their usefulness in the evaluation of methadone-related deaths.

History and Usage of Methadone

Methadone is a potent synthetic opioid that produces both circulatory and respiratory depression. Since it is generally less addictive than heroin and morphine, methadone (also known as Dolophine®) has been clinically used as an analgesic and as a legal alternative for opioid addiction through methadone maintenance treatment (MMT) programs. When used as an analgesic, methadone's effects only last for four to six hours. Methadone's main uses as a painkiller are for chronic pain (most often back pain), cancer, and terminal illnesses. Compared to heroin, methadone has a longer duration of action so it effectively reduces cravings and withdrawal symptoms for up to 72 hours. Additionally, the highs and lows experienced with methadone are less intense than those resulting from heroin (Inaba and Cohen, 2000).

During World War II, methadone was first synthesized in Germany (Baselt and Cravey, 1995). Methadone has been used to treat opioid addiction in the United States since the 1960s when Vincent Dole and Marie Nyswander developed MMT in New York City (Ali and Quigley, 1999; Inaba and Cohen, 2000). This opioid alternative has been shown to decrease the amount of illicit drug use, the spread of the human immunodeficiency virus (HIV) among intravenous drug users, and the amount of criminal activity. Other advantages of methadone include re-employment, social functioning and rehabilitation, and an improved quality of life. Methadone is also less expensive than heroin and other opioids. Moreover, the risks of fatal drug overdose have declined as a result of methadone maintenance (Ali and Quigley, 1999; Wolff, 1998; Toombs and Kral, 2005).

Although methadone is less addictive than heroin, it is unfortunately still abused. There are strict regulations enforced at methadone clinics, but methadone is still sold illicitly. Additionally, patients will often combine methadone with other drugs such as benzodiazepines (e.g. alprazolam), heroin, or illicit methadone to enhance the high (Ali and Quigley, 1999; Inaba and Cohen, 2000). For these reasons, methadone accounts for a high portion of drug deaths each year (Inaba and Cohen, 2000). Wolf et al. (2004) reported a remarkable increase in methadone deaths in Palm Beach County, Florida—from 2 in 1998 to 87 in 2002. Research has identified inadequate dosing as a major cause for patient noncompliance (Ali and Quigley, 1999). A California study spanning 24 years showed that terminating opioid use takes a long time. Those who are continuing to abuse opioids in their late 30s are unlikely to ever cease their use (Wolff, 1998).

Finding the optimal methadone dose for a particular patient is crucial to its success as a maintenance drug. When MMT programs prescribe patients inadequate doses, they will experience withdrawal symptoms, leading them to return to illicit drugs. Withdrawal symptoms include depression, irritability, insomnia, nausea, fatigue, and hot/cold flashes. More severe symptoms include twitching, diarrhea, vomiting, anxiety, fever, and hypertension. Conversely, signs of overmedication are drowsiness, miosis (constriction of the pupil), itching, hypotension, and respiratory depression. When a patient experiences neither withdrawal symptoms nor overmedication symptoms, he or she is considered to be in the therapeutic range (Leavitt et al., 2000).

Routine drug monitoring has been recommended as a means to determine this therapeutic range. This can be done by analyzing serum methadone levels (SMLs). The level of methadone detected in serum shows what is happening to methadone in the body.

In the study by Leavitt et al. (2000), there was no correlation between dose and SMLs. Individual variations in metabolism partially account for this variation. According to this study, doses of 120-700 mg/day may be more effective than the usual 100 mg/day or less. Serum methadone levels cannot be used in isolation when determining a person's optimal methadone dose. The patient's symptoms and clinical state must also be monitored for dosing decisions.

Methadone is available in several forms including liquid, injectable, and tablet. The preferred method is to dissolve it in juice so that it may be taken at a clinic. If a patient cannot come into a clinic one day, there are take-home tablets available, but these often get diverted to the streets (Inaba and Cohen, 2000). Tablets and drinks also run the risk of being consumed by children when inadvertently left out (DiMaio and DiMaio, 1973). The risk of illicit sale is high with injectable methadone as well (Wolff, 1998). A typical starting dosage is 10-20 mg daily. With time, this dose may be increased to 40-100 mg/day with some as high as 180 mg/day (Milroy and Forrest, 2000).

Another problem with methadone is the increased risk of death within the first two weeks of maintenance treatment. This is often due to the dose being increased too quickly, resulting in respiratory depression (Karch and Stephens, 2000). In New South Wales, Caplehorn and Drummer (1999) showed that the risk of accidental toxicity during the first two weeks was 6.7 times that of addicts not in MMT programs. During this time also, the risk is nearly 98 times higher than that of patients who have been in therapy longer than two weeks. This data shows that the first two weeks of treatment are definitely a precarious time. Despite these statistics, it is probable that MMT programs in New South Wales saved 68 lives in 1994 alone.

Metabolism and Excretion

When administered orally, methadone is absorbed quite rapidly into the body. The drug is widely distributed in tissues such as the liver, kidney, lungs, and spleen. In the liver, methadone undergoes biotransformation to the main inactive metabolites 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP) as shown in Figure 1. This process occurs primarily through mono- and di-N-demethylation. These metabolites are then eliminated by the kidney and excreted through the bile, urine, and feces. Smaller amounts of methadol and normethadol are also formed (Jenkins and Cone, 1998; Kerrigan and Goldberger, 2003).

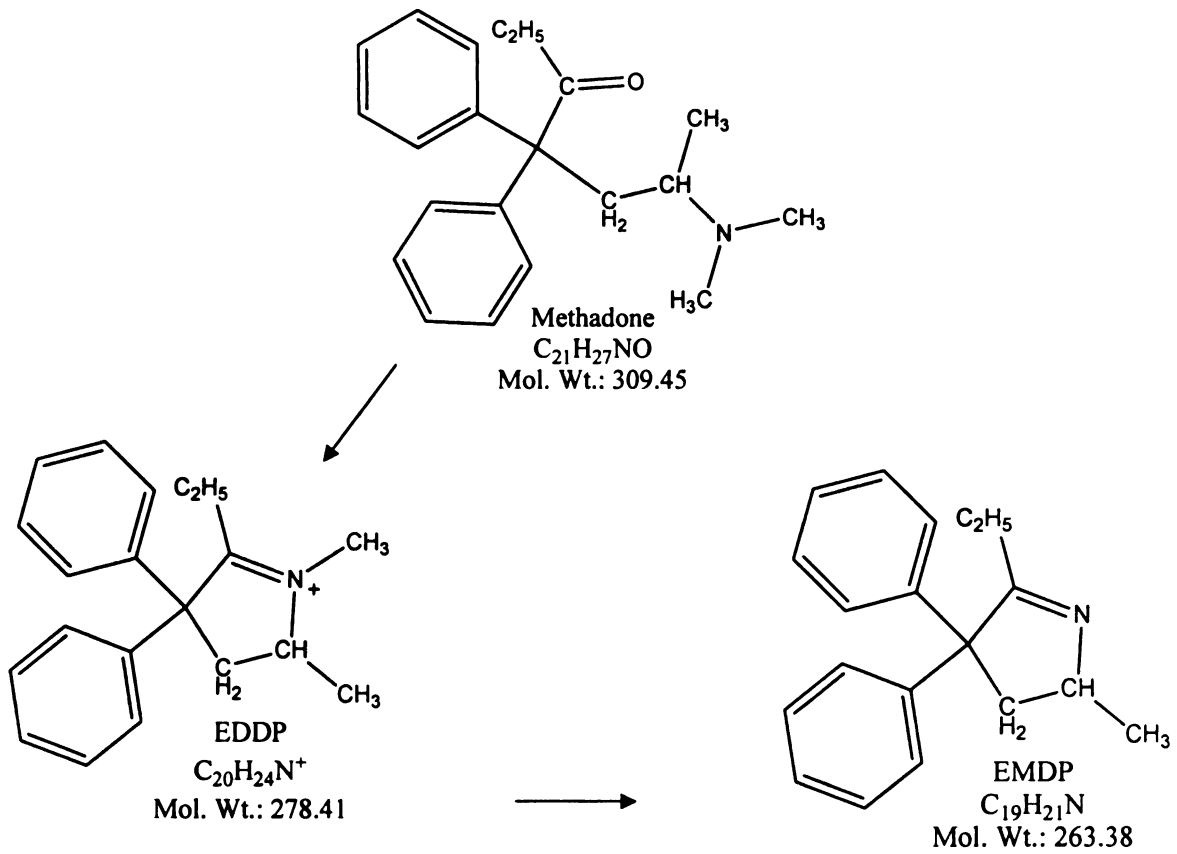

Figure 1. Chemical structures of methadone and its metabolites—EDDP and EMDP.

Methadone, a Schedule II narcotic, is typically dispensed as a 50:50 racemic mixture of the active R-enantiomer and the inactive S-enantiomer (Toombs and Kral, 2005). This basic drug is highly lipophilic with a pK_a value between 8.6 and 9.2. Methadone's volume of distribution (defined as the dose divided by the concentration at equilibrium) is 4-5 L/kg (Baselt and Cravey, 1995; Garrido and Trocóniz, 1999; Karch and Stephens, 2000). Since methadone distributes itself extensively throughout the body (due to its high lipophilicity), it has a long elimination phase. In a study of MMT patients, 5-50% of the dose was excreted as methadone and 3-25% as EDDP. This variation is due to factors such as the dose, metabolic rate, and urine pH. When the urine was acidified, the percentage of unchanged methadone was greater (Baselt and Cravey, 1995).

Methadone has a long elimination half-life that ranges between 15 and 55 hours. This is the main reason why methadone is so useful as a treatment drug. Since its effects are long-lasting, patients normally only have to take it once daily. The half-life of morphine (the primary active metabolite of heroin), on the other hand, is only two to three hours, so patients go through withdrawal only hours after its use (Baselt, 2004). The oral bioavailability of methadone has been reported to range between 0.67 and 0.95 (Garrido and Trocóniz, 1999).

Following oral administration, peak blood concentrations of methadone are attained approximately four hours after dosing. Four hours after a single 15-mg oral dose, a peak plasma concentration of 75 ng/mL has been reported. This concentration slowly declined to 30 ng/mL after 24 hours with a half-life of 15 hours. Peak concentrations in brain occur one to two hours after dosing (Jenkins and Cone, 1998). A

study involving nasal administration revealed that peak plasma concentrations occurred within seven minutes, proving its uptake was very quick (Dale et al., 2002).

Methadone is metabolized by cytochrome P450 (CYP) enzymes in human liver. Iribarne et al. (1996) studied 20 human liver microsomes to determine the involvement of this family of enzymes in the oxidative N-demethylation of methadone. They determined that CYP3A4 is, on average, the principal enzyme involved in this metabolism. Out of nine heterogeneously expressed CYP enzymes, CYP3A4 accounted for 63% of the catalytic activity. Furthermore, this isoform made up 32% of the total CYP group. The other CYP isoforms were CYP1A1, CYP1A2, CYP2C8, CYP2C9, CYP2C18, CYP2D6, CYP3A5, and CYP1E1. Only five of the nine were found to contribute to catalysis—CYP1A1, CYP2C8, CYP2C18, CYP2D6, and CYP3A4. Data published by Wang and DeVane (2003) indicate that CYP3A4, CYP2C8, and CYP2D6 are the three prominent enzymes involved in methadone metabolism. Two other enzymes may also play a role, and they are CYP2C9 and CYP2C19 (Foster et al., 1999).

The CYP3A4 enzyme is inhibited by several mechanism-based inhibitors such as gestodene. Other substances such as quinidine and sulfaphenazole did not inhibit methadone metabolism to the extent of the mechanism-based inhibitors. Inhibition was only 10% for sulfaphenazole—compared to 60-72%, which was seen with the mechanism-based inhibitors (Iribarne et al., 1996). In another study by Oda and Kharasch (2001), it was shown that methadone is metabolized by human intestinal microsomes as well. Methadone undergoes first-pass metabolism in the intestine due to CYP3A4 activity.

Several other factors are thought to affect the metabolism of methadone. For example, people who are chronic alcohol abusers metabolize methadone more slowly. Certain individuals are poor metabolizers because they are deficient in the responsible enzyme. Vitamins, diet, pregnancy, and physical condition may also influence one's metabolism (Leavitt et al., 2000). Studies have shown that methadone concentrations are increased in users who are infected with HIV (Karch and Stephens, 2000). All such people are thus potentially at increased risk for accidental overdose of methadone.

Methadone toxicity is difficult to evaluate due to an overlap in therapeutic and fatal concentrations. One of the main reasons for these coinciding concentrations is tolerance amongst users. Tolerance is defined as an individual's decreased response to a drug following extended use. The body will react to continued drug use in a variety of ways, leading to tolerance. For example, the metabolism and excretion of the drug may increase, and nerve cells may become desensitized. Due to these changes, a user must increase his or her dose to have the same effects. Opioid tolerance occurs quite rapidly and can develop with sustained use (Inaba and Cohen, 2000).

Research has shown that MMT patients have high tolerance in comparison to heroin addicts who are not undergoing treatment. Consequently, an MMT patient can take heroin and survive while a street addict could die from doing so. Even just years after MMT programs were established, lack of opioid tolerance contributed to nearly half of the methadone fatalities (Greene et al., 1974). Tolerance may develop quickly and extensively, but it can be lost quickly, too. There have been instances in which opioid addicts go to prison where their tolerance levels become drastically diminished. Upon being released, they use opioids in the same fashion as they did before going to prison.

Quite often though the results are fatal due to this loss of tolerance (Milroy and Forrest, 2000).

Drug Interactions

Another main factor affecting methadone metabolism is co-medication or drug interactions. As was previously mentioned, methadone users often combine this drug with other substances to enhance the high. Certain P450 enzyme-inducing drugs increase the clearance of methadone. Examples of these drugs include amitriptyline, barbiturates, phenytoin, and rifampicin. Other drugs such as diazepam and cimetidine do just the opposite—they inhibit methadone metabolism and therefore account for increased concentrations of methadone (Oda and Kharasch, 2001). Benzodiazepines such as alprazolam, diazepam, and lorazepam are commonly detected in conjunction with methadone. These, in addition to alcohol, may augment respiratory depression when used in combination with methadone (Barrett et al., 1996; Rogers et al., 1997). A study of oxycodone and hydrocodone fatalities revealed that multiple-drug overdoses are seen with these substances as well (Spiller, 2003).

Pharmacodynamics

Methadone primarily binds to three opioid receptors—mu, kappa, and delta—in the central nervous system (CNS). Another opioid receptor that is less relevant is the sigma receptor. These receptors are primarily located in the brain, spinal cord, and digestive tract (Inaba and Cohen, 2000). Both methadone and morphine are selective to the mu receptor, although morphine's affinity is higher. The mu receptor is primarily

responsible for respiratory depression, euphoria, pain relief, blood pressure, pupil contraction, the gastrointestinal tract, and physical tolerance and dependence. The kappa receptor, on the other hand, generates dysphoria but is also responsible for pain relief and sedation (Garrido and Trocóniz, 1999; Inaba and Cohen, 2000; Kerrigan and Goldberger; 2003). Studies have suggested that tolerance is partly due to changes in mu-receptor function (Garrido and Trocóniz, 1999).

Cross-tolerance is a type of tolerance that results in a reduced response to an opioid following treatment with another one. For instance, a person who has built up tolerance to heroin will also exhibit tolerance to morphine and other opioids. However, it is important to remember that opioids are selective to certain receptors. Thus, a person who has acquired a tolerance to an opioid that works at the mu receptor will not have as much tolerance to a drug that works at a different receptor (Inaba and Cohen, 2000). Research has indicated that methadone does not induce cross-tolerance as readily as morphine (Garrido and Trocóniz, 1999).

In an analysis of 21 methadone deaths in Milwaukee, Wisconsin, the role of pharmacogenomics was assessed. In this study, Wong et al. (2003) studied whether or not CYP2D6 variant alleles were the cause of atypical metabolism, which would lead to methadone toxicity. They found no significant associations between CYP2D6 mutations and methadone toxicity. Therefore, the results of genotyping in this study did not provide any additional information for interpreting the methadone cases and assigning causes and manners of death. According to their article, methadone metabolism involves CYP1A2, CYP3A4, and CYP2D6 enzymes, which differs slightly from those previously discussed.

A study aimed at evaluating methadone's pharmacokinetics and pharmacodynamics involved eight drug-free women who were given a single oral dose. During a four-day period, their vital signs and CYP3A activity were monitored. As part of examining their vital signs, the subjects' eyes were photographed to determine pupil diameters, which indicate pain intensity. All of the subjects' pupils were constricted, which is a common effect of opioids. In addition to measuring CYP3A activity, the concentrations of alpha-1-acid glycoprotein (alpha-1-AGP) were determined (Boulton et al., 2001b; Dale et al., 2002).

Methadone readily binds to this glycoprotein, and Boulton et al. (2001b) concluded that it may function in the first-pass metabolism of R-methadone. While CYP3A and alpha-1-AGP binding can elucidate the activity of R-methadone, these factors do not explain the disposition of S-methadone. For these reasons, this glycoprotein and enzyme may affect the interindividual variability in methadone action. Research has shown that the urinary cortisol ratio (UCR) may also be a marker for CYP3A activity. Following oral dosage of methadone, UCR declines, which suggests that methadone inhibits CYP3A (Boulton et al., 2001a).

Postmortem Redistribution

In addition to tolerance, another phenomenon that complicates interpretation of methadone toxicity is known as postmortem redistribution. This is the process by which drugs move between bodily fluids and tissues after death. They essentially diffuse from areas of higher concentration to areas of lower concentration after cellular membranes have been disrupted (Cook et al., 2000; Drummer, 2004). Drugs that are highly lipophilic

and have increased concentrations in tissues are more susceptible to redistribution. Drummer (2004) evaluated 23 different drugs/drug classes based on their extent of redistribution. The extent was categorized as either low, low to moderate, or moderate. Of these three, methadone's extent of redistribution was classified as moderate. This makes sense considering methadone is a highly lipid soluble drug with a large volume of distribution.

If the gastrointestinal tract contains large amounts of unabsorbed drug, this could leak to surrounding tissues and fluids. Analyses of heart blood in the central region and peripheral blood (such as that acquired from the femoral vein) have shown that the latter is less subject to contamination. Peripheral blood (femoral) is thus the preferred specimen for toxicological analysis the majority of the time. Due to the perplexing factor of postmortem redistribution, it is important for toxicologists to know the approximate timing of specimen collection relative to the time of death (Cook et al., 2000).

A case at the OCME in Baltimore, Maryland prompted Levine et al. (1995) to study 15 methadone cases to evaluate site dependence of this drug. The methadone concentrations in this initial case were 2.4 mg/L in heart blood and 0.8 mg/L in subclavian blood. In addition to testing heart blood, an alternative sample such as subclavian blood was analyzed for each case. They then compared the ratios of these samples and found variation of concentrations. Only four of the 15 cases had results within 20% of each other. From their study, they could not conclude that one sample would always have a higher concentration than another or that one sample was preferential to another.

Milroy and Forrest (2000) also examined postmortem site dependence and drug redistribution of methadone. Of their 111 cases, gastric contents were analyzed for 94 of them. Furthermore, multiple site sampling was performed for 26 of the cases. This is the process of collecting peripheral blood in both the arm and leg and comparing their methadone concentrations. They found extreme unpredictable variation in concentrations from the different sites, suggesting redistribution.

Toxicological Analysis

A variety of acceptable toxicological analysis schemes for methadone have been reported in the literature. In addition to the method of gas chromatography/mass spectrometry (GC/MS) utilized herein, high-performance liquid chromatography (HPLC) has been used as well as gas chromatography with nitrogen-phosphorus detection (GC-NPD). The most common type of extraction used for methadone analysis is solid-phase, although liquid-liquid extraction has been documented as well (Levine et al., 1995; Rio et al., 1987). When methadone toxicity is suspected, pathologists nearly always perform a full autopsy and collect a combination of the following samples: heart blood, peripheral blood, vitreous humor, brain, liver, kidney, gastric contents, urine, and bile (Drummer, 2004). Of these samples, blood, liver, and urine are the most common specimens analyzed in drug-suspected cases. Due to postmortem redistribution, it is important for medical examiners to collect two different blood specimens (one from the central region and one from a peripheral site) so that their concentrations may be compared. In addition to redistribution, there are other difficulties of working with postmortem samples, especially in cases of decomposition. These oily samples are useful for drug screening,

but they are not favorable for quantitative analysis. In instances of severe decomposition, skeletal muscle is oftentimes the only available specimen for collection (Drummer, 2004).

Blood is definitely a useful indicator of drug toxicity. Since metabolism primarily occurs in the liver, results from this tissue can be valuable as well. Analysis of vitreous humor has provided the detection of drugs, although this type of testing is especially useful for the quantitation of ethanol and other volatiles. Since many drugs act on the brain, toxicologists have analyzed this tissue for drug concentrations. However, these results are often difficult to interpret due to the uneven distribution of drugs there. Gastric contents function mainly for determining time and type of drug administration (Drummer, 2004).

Karch and Stephens (2000) reviewed 38 methadone-related deaths at the San Francisco Medical Examiner's Office. The average concentration of methadone in blood for these cases was 957 ng/mL, and that for EDDP was 253 ng/mL. The mean ratio of parent drug to EDDP was 13.6 with a range of 0.572 to 60. Diazepam was the most common drug detected in addition to methadone. They also reported that methadone concentrations were increased in HIV patients.

Baselt and Cravey (1995) report methadone concentrations in fatalities for various specimens. The average concentrations in blood and brain were 1.0 mg/L and 1.0 mg/kg, respectively, and that in kidney was 2.9 mg/kg. Liver was the tissue with the highest concentration of methadone at 3.8 mg/kg. Other reported methadone concentrations ranged from 0.18-3.99 mg/L for deaths caused or related to drug toxicity. In this same study, the range for deaths not related to methadone toxicity was 0.18-3.03 mg/L

(Gagajewski and Apple, 2003). For deaths attributable to methadone overdosage in a separate study, the listed range was 0.114-1.939 mg/L. For those due to trauma, the range was 0.072-2.7 mg/L. The maximum concentration here is much higher than that from the former group, and again there is an overlap (Wolf et al., 2004).

EXPERIMENTAL

Specimens

The cases included in this study were all methadone-positive postmortem cases received at the State of Delaware OCME during a three and a half year period—from September 2001 through the beginning of March 2005. All specimens collected during autopsy (with the exception of bile and urine) were analyzed for methadone and its primary metabolite (EDDP). When available, the following specimens were analyzed: vitreous humor, peripheral blood (femoral), heart blood, brain, gastric contents, liver, and kidney. In some instances, antemortem blood or serum specimens were also available for analysis. Table 1 shows all of the specimens that were analyzed for each of the 100 cases. Following collection at autopsy, specimens were placed into refrigerated storage at approximately 5°C. Specimens were later transferred to a freezer for long-term storage (approximately -10 to -12°C).

One milliliter of blood or vitreous humor was required for analysis. Solid tissue specimens such as brain, liver, and kidney were homogenized with deionized/distilled (DI) water to prepare a 1:4 tissue homogenate for analysis. All gastric contents were initially prepared at a 1:25 dilution. If the initial results fell outside of the linear range of the calibration curve, specimens were reanalyzed at an appropriate dilution. All dilutions were prepared with DI water.

Table 1. Table showing all of the specimens that were analyzed for each case.

Case	Specimens
1	HB
2	HB
3	Blood
4	Cavity Fluid
5	HB
6	Blood
7	HB
8	Ante Blood
9	HB
10	Blood
11	PB(Fem)
12	HB
13	HB
14	HB
15	HB
16	HB, Liver
17	HB
18	Aorta Blood
19	PB(Fem), HB
20	PB(Fem)
21	HB
22	HB
23	Ante Blood
24	HB
25	HB
26	PB(Fem)
27	PB(Sub)
28	PB(Sub)
29	PB(Fem)
30	VH, PB(Fem), HB, Brain, GC, Liver
31	VH, HB, Brain, GC, Liver
32	VH, PB(Fem), HB, Brain, GC, Liver
33	VH, PB(Fem), HB, Brain, GC, Liver, Kidney
34	VH, PB(Fem), HB, Brain, GC, Liver
35	VH, HB, Liver
36	VH, PB(Fem), PB(Sub)
37	VH, PB(Fem), HB, Brain, GC, Liver
38	VH, PB(Fem), HB, Brain, GC, Liver, Kidney
39	VH, PB(Fem), HB, Brain, GC, Liver
40	VH, PB(Fem), Brain, Liver, PB(Sub)
41	VH, PB(Fem), HB, GC, Liver
42	VH, PB(Fem), HB, Brain, GC, Liver
43	VH, PB(Fem), HB, Brain, GC, Liver
44	VH, PB(Fem), HB, Brain, GC, Liver
45	VH, PB(Fem), HB, GC
46	Brain, GC, Liver
47	HB, Brain, Liver
48	VH, PB(Fem), HB, GC, Liver
49	VH, PB(Fem), HB
50	VH, PB(Fem), HB, Brain, GC, Liver

Table 1 (cont'd).

Case	Specimens
51	VH, PB(Fem), HB, Brain, GC, Liver
52	VH, PB(Fem)
53	VH, PB(Fem), HB, Brain, GC, Liver
54	VH, HB, GC, Liver, PB(Sub)
55	VH, HB, Brain, GC, Liver, PB(Sub)
56	VH, HB, Brain, GC, Liver, PB(Sub)
57	VH, PB(Fem), HB, Brain, GC, Liver
58	VH, PB(Fem), HB, Brain
59	VH, HB, Brain, GC, Liver
60	VH, PB(Sub)
61	VH, HB
62	VH
63	VH, PB(Fem), HB, Brain, GC, Liver
64	VH, PB(Fem), HB
65	VH, PB(Fem), HB, Brain, GC, Liver
66	VH, PB(Fem), HB, Brain, GC, Liver
67	VH, PB(Fem), HB, Brain, GC, Liver
68	VH, PB(Fem), HB, Brain, GC, Liver
69	VH, HB, Brain, GC, Liver, Blood
70	VH, HB
71	Ante Blood, Ante Serum
72	VH, PB(Fem), HB, Brain, GC, Liver
73	VH, PB(Fem), HB, Brain, GC, Liver
74	VH, PB(Fem), HB, Brain, GC, Liver
75	VH, PB(Fem), HB
76	VH, PB(Fem), HB, GC, Liver
77	VH, PB(Fem), PB(Sub)
78	VH, PB(Fem), HB, Brain, GC, Liver
79	VH, PB(Fem), HB, Brain, GC, Liver
80	PB(Fem), HB, Brain, GC, Liver
81	Ante Blood, Ante Serum
82	VH, PB(Fem), HB, Brain
83	PB(Fem), HB, Brain, GC, Liver
84	VH, PB(Fem), HB, Liver
85	HB
86	VH, PB(Fem), HB, Brain, GC, Liver
87	VH, PB(Fem), HB, Brain, GC, Liver
88	Brain, GC, Liver, Kidney
89	VH, PB(Fem), HB, Brain, GC, Liver
90	VH, Brain, PB(Sub)
91	VH, PB(Fem), HB, Brain, Liver
92	PB(Fem), HB, Liver
93	VH, PB(Fem), HB, Brain, GC, Liver
94	VH, PB(Fem), HB, Brain, GC, Liver
95	VH, PB(Fem), HB, Brain, GC, Liver, Kidney
96	VH, PB(Fem), PB(Sub)
97	VH, PB(Fem), HB, Brain, GC, Liver
98	VH, PB(Fem), HB, Brain, GC, Liver
99	VH, PB(Fem), HB, Brain, Liver, Kidney, Ante Blood, Ante Serum
100	VH, HB

Chemicals and Materials

(±)-Methadone, (±)-Methadone-d_3, EDDP, and EDDP-d_3 standards were purchased from Cerilliant® (Round Rock, Texas). The following reagents were required as well: methanol, sodium phosphate monobasic, sodium phosphate dibasic, glacial acetic acid, methylene chloride, isopropanol, concentrated ammonium hydroxide, and ethyl acetate (absolute). All reagents were ACS Grade and purchased from Fisher Scientific (Pittsburgh, Pennsylvania). Solid-phase extraction columns (ZSDAU020) were purchased from United Chemical Technologies (UCT), Inc. (Bristol, Pennsylvania).

Extraction

For each run, a matrix blank and six calibrators containing both methadone and EDDP were prepared (using calibrated pipettes) in properly labeled 16 x 125 mm test tubes. The calibrators were prepared in drug-free whole blood and ranged in concentration from 25 ng/mL to 1000 ng/mL. Two whole blood controls were prepared at concentrations of 100 and 500 ng/mL. Case specimens were prepared in 1-mL aliquots. Solid tissue specimens were prepared in duplicate, and one aliquot was spiked with 500 ng of methadone and 500 ng of EDDP. The spiked tissue specimens were prepared so the method of standard addition could be used to ensure that there were no significant matrix effects on the quantitation of methadone and EDDP in solid tissue specimens (Andollo, 1998). Internal standard containing both d_3-methadone and d_3-EDDP (100 ng each) was added to all calibrators, controls, and case specimens. All samples were then mixed with 4 mL DI water and 2 mL phosphate buffer (100 mM, pH

6.0) and vortex-mixed for 15 seconds. The samples then sat at room temperature for 5 minutes followed by centrifugation at 3000 RPM for 10 minutes.

Solid-phase extraction of methadone and EDDP was performed with combination reverse phase/cation exchange columns using a method adopted from UCT, Inc. (Moore, 1994). The SPE columns were conditioned with 3 mL methanol, 3 mL DI water, and 2 mL phosphate buffer. The supernatant of each sample was then applied to a column, allowing gravity flow to pull it through. The columns were washed with 3 mL DI water, 1.25 mL 100 mM acetic acid, and 3 mL methanol, respectively. Following this, the columns were dried for 5 minutes under vacuum (>10 inches Hg).

The analytes were eluted with 3 mL Basic Drug Elution Solvent (containing methylene chloride/isopropanol/ammonium hydroxide—78/20/2) into properly labeled 5-mL conical centrifuge tubes. Extracts were evaporated to dryness at approximately 35°C under nitrogen in an adjustable temperature evaporator and then reconstituted in 50 µL ethyl acetate. Final extracts were transferred to autosampler vials with reduced volume inserts, and a 1-µL aliquot was injected into the gas chromatograph for analysis.

Instrumentation

Analysis of methadone and EDDP was performed on a Hewlett Packard (HP) model 6890 gas chromatograph (GC) with an Agilent 5973 Network Mass Selective (MS) Detector (Wilmington, Delaware). An HP5-MS column (30 m length x 250 µm diameter x 0.25 µm film thickness) was used for separation. The GC was operated in the constant pressure mode; the pressure was variable to maintain a constant retention time for methadone. The GC oven temperature program started at 90°C where it was held for

1 minute, then ramped to 200°C at 25°C/min, increased and then ramped to a final temperature of 300°C at 20°C/min where it was held for 2 minutes for a total run time of 12.40 minutes. The GC inlet mode was pulsed splitless with an inlet temperature of 260°C. The purge flow was 50 mL/min with a purge time of 1.00 minute. Ultrapure helium was the carrier gas.

The mass spectrometer was operated in the selected ion monitoring (SIM) mode. The transfer line temperature was set to 280°C. The ions monitored for d_3-EDDP and EDDP were 276, 277, 278, 279, 280, and 281. The ions monitored for d_3-methadone and methadone were 223, 226, 294, 297, 309, and 312.

Chromatography and Mass Spectrometry

Figure 2 is a total ion chromatogram of EDDP and methadone, showing that EDDP elutes first. Figures 3-6 are ion chromatograms and corresponding SIM mass spectra of d_3-EDDP, EDDP, d_3-methadone, and methadone, respectively. These figures illustrate the peaks and ions used to identify and quantitate the analytes for each case. As Figures 3 and 4 show, the retention time for d_3-EDDP was 8.47 min, and that for EDDP was 8.49 min. The retention time for d_3-methadone was 9.00 min, and that for methadone was 9.02 min (Figures 5 and 6). The deuterated internal standards (which contain three deuterium atoms in place of three hydrogen atoms) were identified by ions that were three atomic mass units heavier than those seen with EDDP and methadone. Ethyl acetate was used as a solvent blank between standards, controls, and case specimens where necessary. An ion chromatogram of ethyl acetate is also shown as Figure 7. There was no carryover present in any of the blanks.

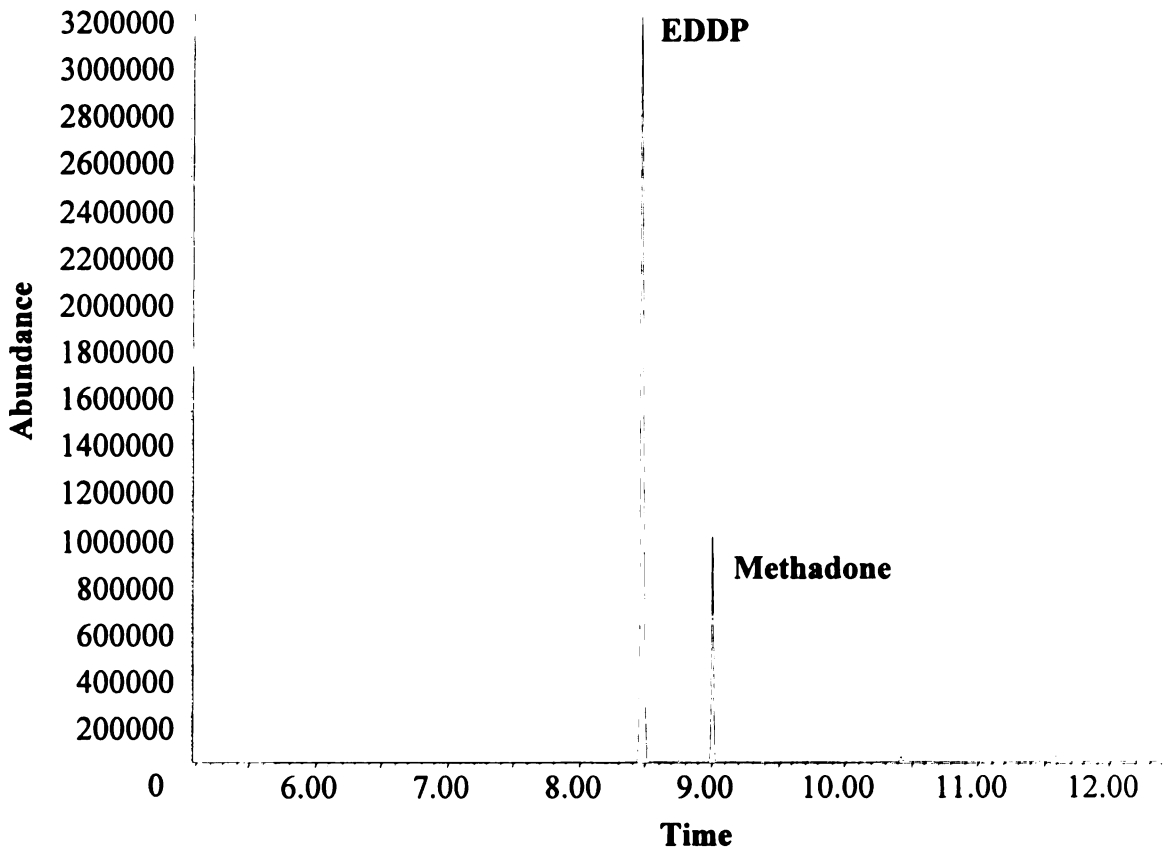

Figure 2. Total ion chromatogram of EDDP and methadone.

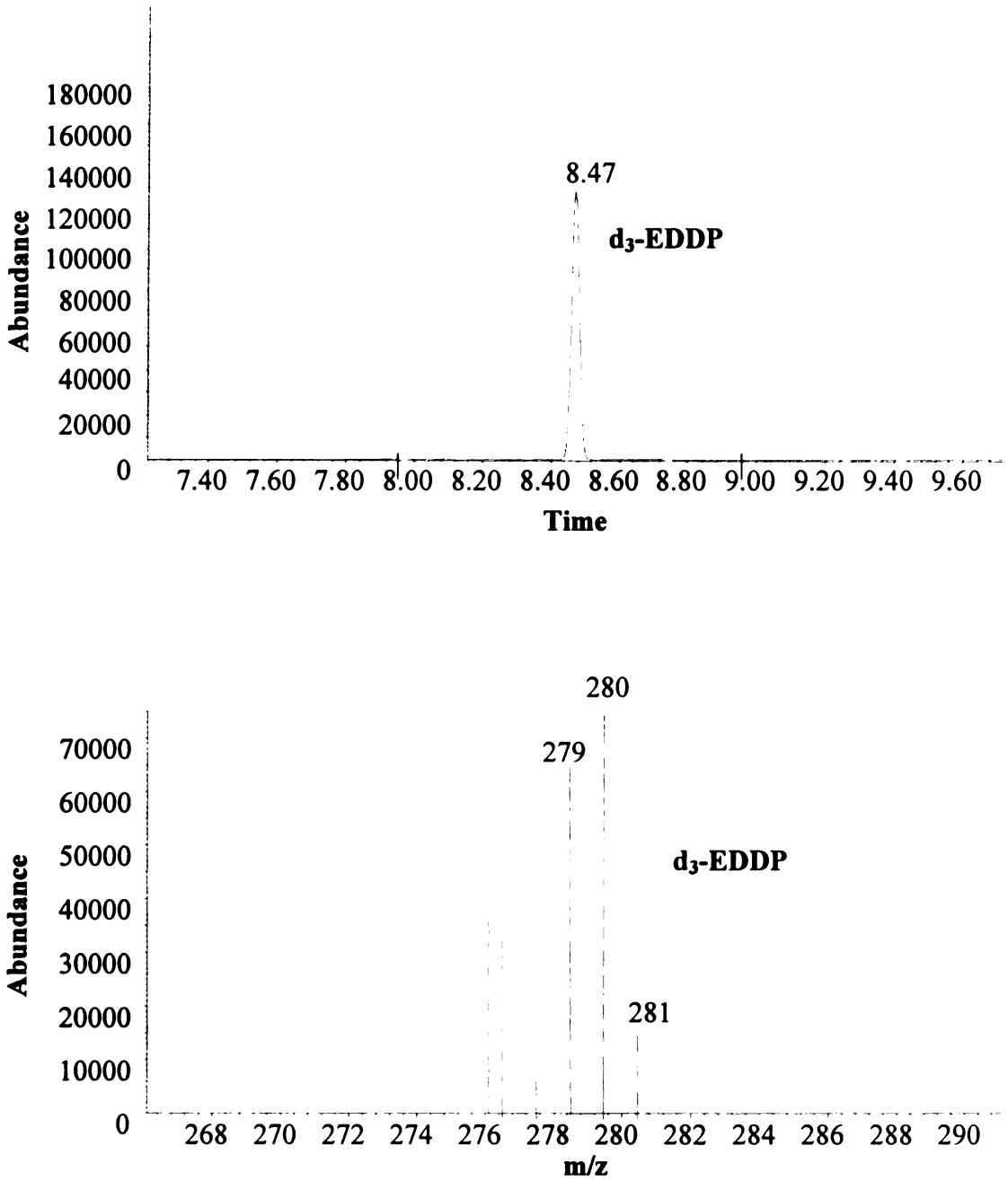

Figure 3. Ion chromatogram (top) and SIM mass spectrum (bottom) of d_3-EDDP.

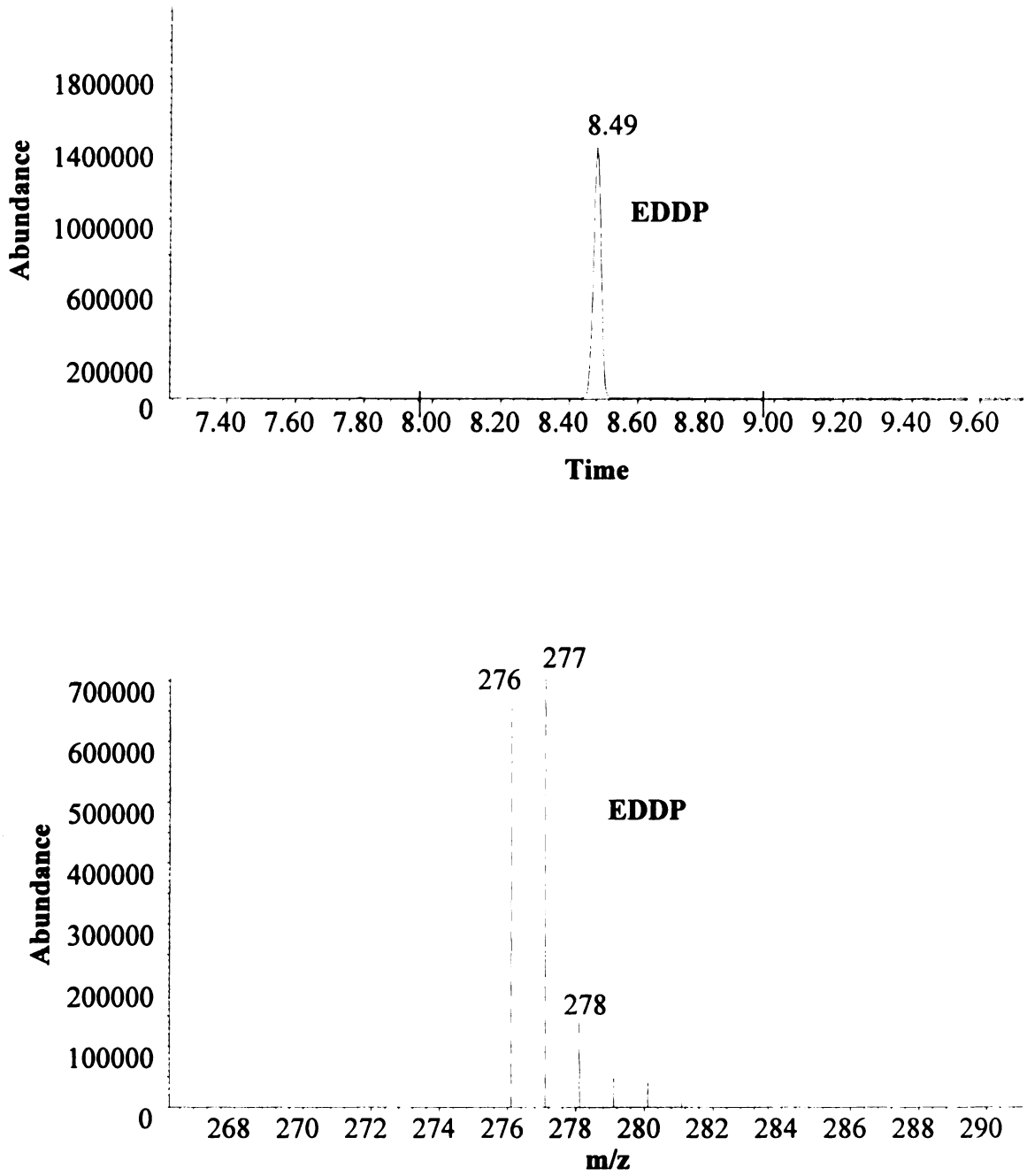

Figure 4. Ion chromatogram (top) and SIM mass spectrum (bottom) of EDDP.

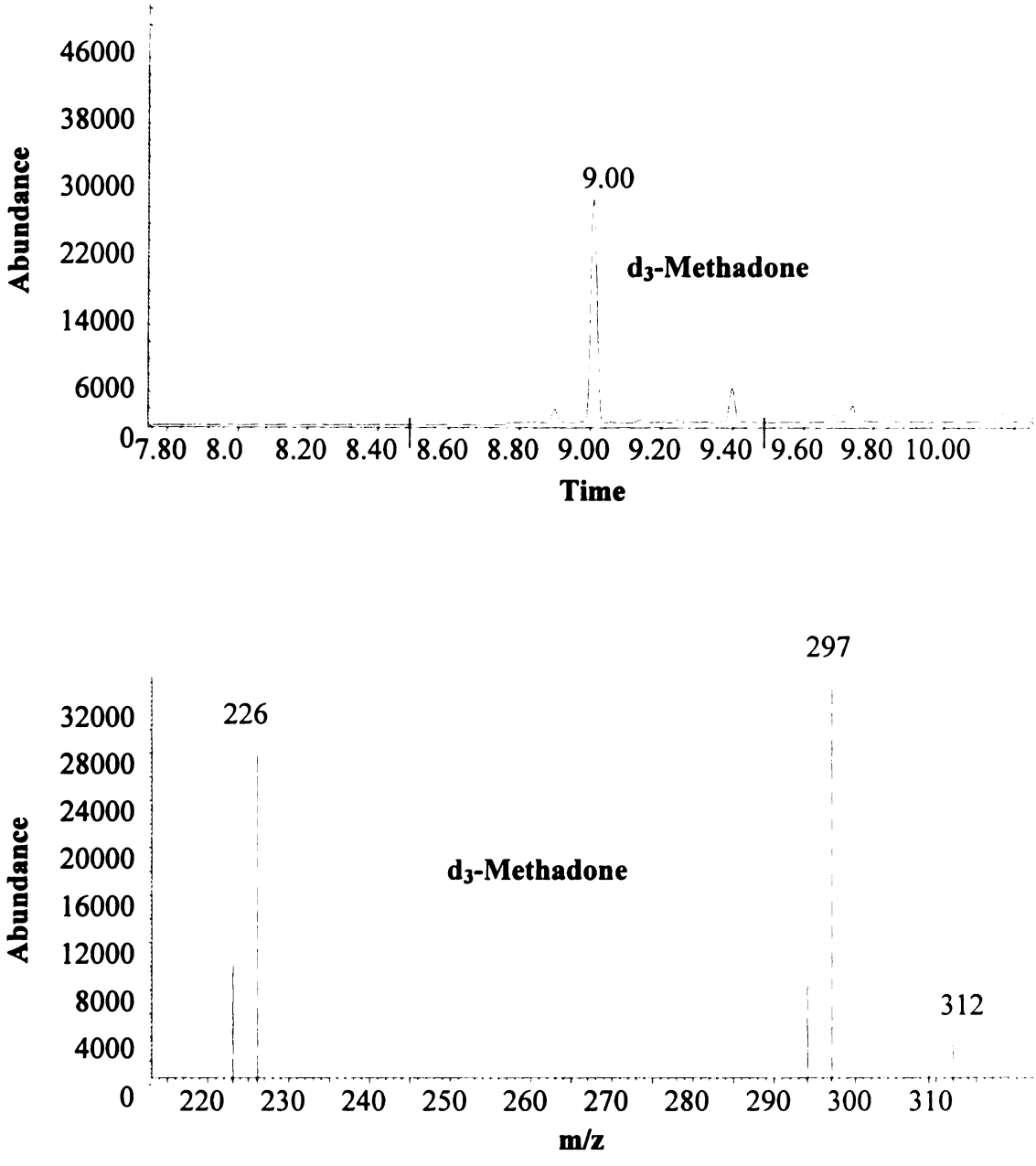

Figure 5. Ion chromatogram (top) and SIM mass spectrum (bottom) of d_3-methadone.

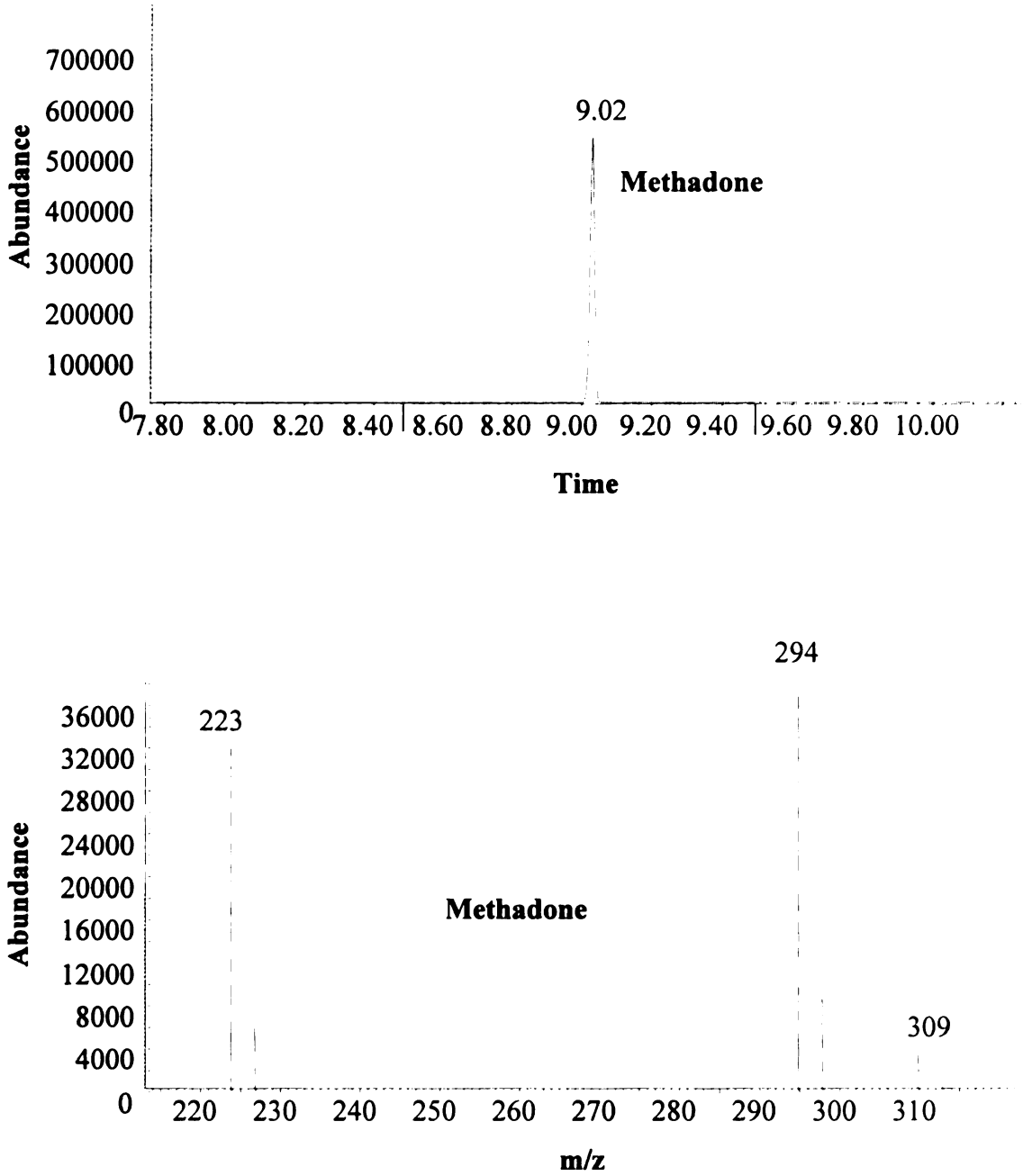

Figure 6. Ion chromatogram (top) and SIM mass spectrum (bottom) of methadone.

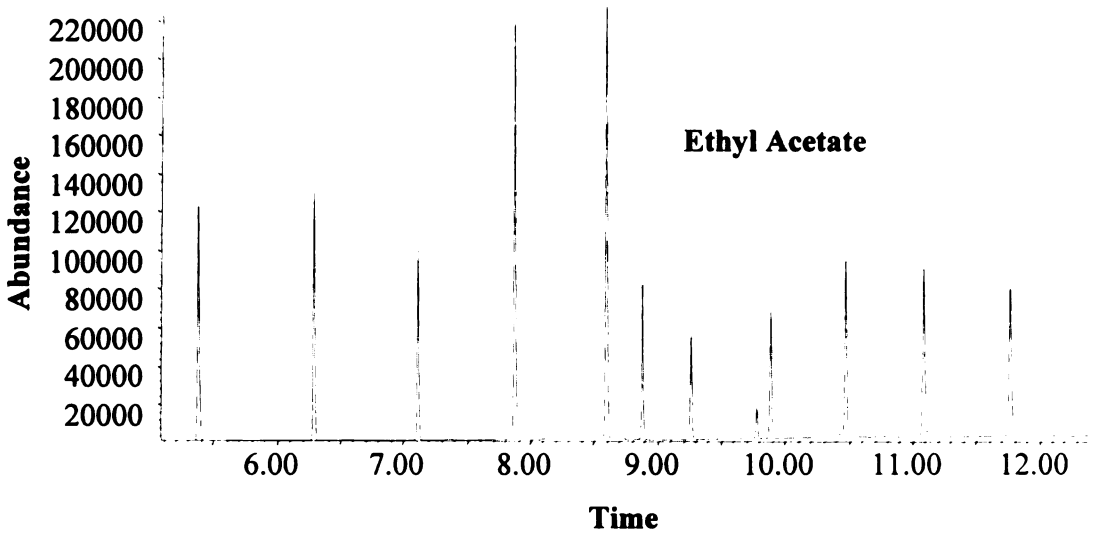

Figure 7. Ion chromatogram of ethyl acetate, which was used as a solvent blank.

Quantitation and Acceptability

A multi-point linear calibration curve containing seven points was constructed for each separate analytical batch. Each calibration curve had a linear correlation coefficient of 0.985 or better. All data files were quantitated, and calculations were based on the internal standardization method. The internal standards were detected in all samples. The limits of quantitation for methadone and EDDP were 25 ng/mL and 50 ng/mL, respectively. Quantitative results for all calibrators were within ±20% of their expected values. Qualifier ion ratios were also evaluated. The ratios of positive findings were required to be within 20% of the ion area ratios of a calibrator of similar concentration. All quantitative results for the controls were also within ±20% of their target values.

RESULTS AND DISCUSSION

This study included 100 methadone-related deaths that were identified during a three and a half year period—September 6, 2001 through March 1, 2005—in the small state of Delaware. Of the total, 61 were male and 39 were female. Eighty-one percent were Caucasian, 18% were African American, and only one person was Hispanic. The average age of the decedents was 41 years old (with a range of 16-65). These statistics are very similar to those reported from other states.

In a study by Wolf et al. (2004) of 125 methadone-related deaths in Palm Beach County, Florida, 98 (78%) were males. The average age was 39 years old, and all but one of their decedents was Caucasian. Similarly, in Hennepin County, Minnesota, over 90% of the methadone decedents from 1992 to 2002 were Caucasian, nearly 80% were male, and the average age was 45 years old (Gagajewski and Apple, 2003). These same figures were seen in the past as well. A Harris County, Texas study from 1987 to 1992 revealed that 77% of the methadone decedents were male, 85% were Caucasian, and the median age was 35 years old (Barrett et al., 1996).

For this study, investigative reports were reviewed for case histories including any known methadone usage, and toxicology reports were examined to determine additional positive drug findings. Additional drugs were detected in approximately 70% of the 95 completed cases. Eighteen decedents were positive for ethanol. Eight decedents had alprazolam in their systems, nine had diazepam, and 12 had nordiazepam (the major metabolite of diazepam). Alprazolam and diazepam are benzodiazepine tranquilizers that are primarily used to treat anxiety disorders. Cocaine was detected in ten cases, and

benzoylecgonine and ecgonine methyl ester (metabolites of cocaine) were found in 23% and 20% of the 100 cases examined, respectively.

Autopsy reports were also reviewed for the causes and manners of death in each case. The manner of death in over half the cases was accident, which accounted for 54 cases. Of the remaining 46 cases, 26 were classified as natural deaths, seven were classified as suicides, four were undetermined, and nine are still pending. From this information, the cases were categorized into groups as follows:

Group 1A) Drug-related deaths in which death was due to methadone only;

Group 1B) Drug-related deaths in which death was due to methadone in combination with other drugs;

Group 2) Drug-related deaths in which methadone was not a contributing factor;

Group 3) Deaths in which methadone was an incidental finding (e.g. deaths due to trauma);

Group 4) Natural deaths that were aggravated by methadone; and

Group 5) Undetermined or pending cases.

Of the 100 cases, 13 were placed in Group 1A, 27 in Group 1B, 6 in Group 2, 33 in Group 3, 9 in Group 4, and 12 in Group 5. Table A1, which can be found in the Appendix, is a compilation of all this information. As the table shows, there were several frequent findings. These include a history of heroin, cocaine, alcohol, and/or prescription medication abuse, a history of depression and/or suicide attempts, back pain, Hepatitis C,

cirrhosis, HIV, and pneumonia. Karch and Stephens (2000) listed similar findings such as cirrhosis, pneumonia, and HIV.

Methadone and EDDP Concentrations

All available specimens from each case were analyzed. Following GC/MS analysis, the methadone and EDDP concentrations were determined. These results have been categorized in tables according to the various groups so that trends in concentrations could be evaluated. In all subsequent tables, any blank spaces indicate that a given specimen was either not analyzed (due to an insufficient quantity) or not received. The average, standard deviation (SD), number of positive cases (n), and range is included for each category as well. An asterisk next to a case number indicates that additional specimens such as antemortem blood were analyzed.

Group 1A

The concentrations for Group 1A (drug deaths attributable to methadone only) are shown on the next page in Table 2. As the table shows, the average concentration of methadone in vitreous humor was 260 ng/mL (n = 10). EDDP was detected in vitreous humor in only one case (Case 82) in this group. This case also happened to have the highest methadone concentration detected in this specimen (840 ng/mL) amongst all six groups. This analyte was only detected in one other vitreous sample from Group 1B. The peripheral blood (femoral) concentrations ranged from 330-1800 ng/mL for methadone with an average of 950 ng/mL (n = 9; SD = 510). The average concentration of EDDP in peripheral blood was 110 ng/mL with a range of 50-220 ng/mL (n = 8; SD = 54).

Table 2. Concentrations of vitreous humor, peripheral blood (femoral), heart blood, brain, gastric contents (total amount), and liver for each case in Group 1A. Averages, standard deviations, number of positive cases (n), and ranges are also included.

Case	VH (ng/mL)		PB(Fem) (ng/mL)		HB (ng/mL)		Brain (ng/g)		GC (mg)		Liver (ng/g)	
	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN
1					ND	300						
15					ND	130						
25					ND	350						
36 *	ND	680	120	1400								
50	ND	110	92	460	ND	250	ND	1300	0.18	7.2	970	5000
59	ND	110			86	640	ND	1600	ND	1.0	700	4100
65	ND	150	64	610	77	570	ND	1300	ND	0.59	720	4800
66	ND	60	ND	330	ND	240	ND	580	ND	ND	ND	1500
68	ND	300	110	1800	85	1500	ND	2700	0.11	2.7	900	4800
74	ND	120	50	1200	ND	800	ND	2100	ND	0.42	360	3700
76	ND	170	140	1100	130	1000			0.045	4.2	790	5600
82	120	840	220	1200	100	670	ND	3500				
89	ND	84	73	440	96	600	ND	1000	ND	0.34	310	2600
Average	**NA**	**260**	**110**	**950**	**96**	**590**	**NA**	**1800**	**0.11**	**2.3**	**680**	**4000**
SD	**NA**	**270**	**54**	**510**	**19**	**390**	**NA**	**960**	**0.069**	**2.6**	**250**	**1400**
n	**1**	**10**	**8**	**9**	**6**	**12**	**0**	**8**	**3**	**7**	7	**8**
Range	**NA**	**60-840**	**50-220**	**330-1800**	**77-130**	**130-1500**	**NA**	**580-3500**	**0.045-0.18**	**0.34-7.2**	**310-970**	**1500-5600**

* Additional specimens analyzed

In a study of 125 methadone-related deaths in Palm Beach County, Florida, the deaths were grouped as follows: methadone toxicity, combined drug toxicity, other drugs, natural causes, and trauma. These groupings are very similar to those chosen for this project. In deaths attributable to methadone toxicity alone, the mean concentration of methadone in peripheral blood was 559 ng/mL with a range of 114-1939 ng/mL (Wolf et al., 2004). The average in this study for Group 1A was significantly higher. In another study by Milroy and Forrest (2000), the average concentration of methadone for a category in which methadone was the only drug involved was 584 ng/mL, which is again less than that seen in this project. The case with the highest reported methadone concentration in this group was Case 68. Referring back to Table A1, it is interesting to see that this was the only case in this group in which the manner of death was suicide (intentional overdose). All others were accidents.

In heart blood, the mean concentration of EDDP was 96 ng/mL (n = 6). That for methadone was 590 ng/mL (n = 12). The case with the maximum methadone concentration was again Case 68. EDDP was not detected in brain for any of the cases. The average concentration of methadone in this tissue was 1800 ng/g (n = 8). The highest concentration was not seen with Case 68 this time but with Case 82 (Caucasian female on methadone for chronic back pain).

The average total amount of methadone in the gastric contents was 2.3 mg (n = 7). The highest total amount (7.2 mg) was seen for Case 50, which was deemed an accident. Gastric contents can be particularly useful to establish route of administration or a minimum dose administered. However, one must ensure that the entire gastric contents is collected and that it is homogenized prior to analysis to obtain a reliable result. The

concentration range for liver was 310-970 ng/g for EDDP (n = 7; mean = 680; SD = 250) and 1500-5600 ng/g for methadone (n = 8; average = 4000; SD = 1400). Case 76 had the highest concentration of methadone, and this was another person who was taking methadone for back pain. He could have been taking the drug for an extended time, which would cause it to accumulate in his body.

Group 1B

Table 3 provides concentrations of the various specimens for cases in Group 1B (multiple drug deaths involving methadone). The mean concentration of methadone in vitreous was 160 ng/mL (n = 16). The average concentration of methadone in peripheral blood was 640 ng/mL (n = 16; SD = 370; range = 170-1500). The average for EDDP was 180 ng/mL (n = 10; SD = 130; range = 64-450). Referring back to the study by Wolf et al. (2004), the average concentration of methadone in deaths due to combined drug toxicity was 411 ng/mL. Again, the average for this study is higher. Case 49 had the concentration of 1500 ng/mL, which was the maximum for this group. It is worthy to note that this person was HIV positive. As was already mentioned, research has indicated that methadone concentrations tend to be high in individuals infected with HIV (Karch and Stephens, 2000).

The mean EDDP concentration in heart blood was 190 ng/mL (n = 11). For methadone, the average was 530 ng/mL (n = 20). The average concentration of methadone in brain was 1300 ng/g (n = 13). The maximum concentration of 2900 ng/g was seen with a person who was on methadone up to three times a day for chronic back pain (Case 34). This case also had the highest total amount of methadone in the gastric contents (5.9 mg). The average was 0.80 mg (n = 14). The average concentration of

Table 3. Concentrations of vitreous humor, peripheral blood (femoral), heart blood, brain, gastric contents (total amount), and liver for each case in Group 1B. Averages, standard deviations, number of positive cases (n), and ranges are also included.

Case	VH (ng/mL)		PB(Fem) (ng/mL)		HB (ng/mL)		Brain (ng/g)		GC (mg)		Liver (ng/g)	
	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN
7					32	520						
8 *												
17					130	610						
18 *												
19			66	400	140	600						
20			ND	1200								
22					69	160						
23 *												
29			180	1100								
30	ND	47	ND	230	ND	350	ND	500	ND	0.83	ND	990
32	ND	66	ND	500	ND	400	ND	1100	ND	0.71	320	3800
34	ND	300	73	690	75	660	ND	2900	0.34	5.9	590	5800
35	ND	110			ND	92					500	1500
41	ND	120	98	540	67	460			ND	0.010	600	3000
43	ND	160	ND	740	ND	630	ND	1500	ND	0.015	680	5500
46							ND	810	ND	0.13	320	2800
49	ND	410	280	1500	270	1300						
51	ND	110	ND	360	ND	440	ND	1100	ND	0.56	330	3600
53	ND	120	ND	700	900	1100	ND	1300	ND	1.3	530	3900
54 *	ND	72			ND	250			ND	0.15	250	2200
55 *	ND	240			ND	150	ND	1100	ND	0.21	220	2900
56 *	ND	130			ND	230	ND	980	ND	0.39	530	3100
73	ND	56	300	350	ND	190	ND	490	ND	0.45	470	3700
77 *	ND	110	450	170								
83			64	630	86	780	ND	1900	ND	0.43	580	3800
86	ND	97	65	280	65	230	ND	1000	ND	0.16	530	1200
91	65	470	270	890	250	1400	ND	1900			1500	9200
Average	**NA**	**160**	**180**	**640**	**190**	**530**	**NA**	**1300**	**NA**	**0.80**	**530**	**3600**
SD	**NA**	**130**	**130**	**370**	**250**	**380**	**NA**	**660**	**NA**	**1.5**	**300**	**2000**
n	**1**	**16**	**10**	**16**	**11**	**20**	**0**	**13**	**1**	**14**	**15**	**16**
Range	**NA**	**47-470**	**64-450**	**170-1500**	**32-900**	**92-1400**	**NA**	**490-2900**	**NA**	**0.010-5.9**	**220-1500**	**990-9200**

* Additional specimens analyzed

EDDP in liver was 530 ng/g (n = 15; SD = 300; range = 220-1500). That for methadone was 3600 ng/g (n = 16; SD = 2000; range = 990-9200). The decedent with the maximum methadone concentration here (Case 91) was known to go to the methadone clinic daily for heroin abuse.

Group 2

Group 2 consists of drug deaths in which methadone was not contributory. Table 4 contains concentrations for cases in this group. The mean concentration of methadone in vitreous was 110 ng/mL (n = 3). Peripheral blood had an average methadone concentration of 410 ng/mL (n = 3; SD = 290; range = 82-600). Averages for heart blood were as follows: EDDP—60 ng/mL (n = 2) and methadone—480 ng/mL (n = 4).

Methadone was detected in the brain at an average amount of 1000 ng/g (n = 3). The average total amount of EDDP in gastric contents was 0.017 mg (n = 2), and that for methadone was 1.0 mg (n = 3). Liver had an average EDDP concentration of 570 ng/g with a range of 400-780 ng/g (n = 3; SD = 190). The mean methadone concentration in liver was 5000 ng/g with a range of 2800-7900 ng/g (n = 3; SD = 2600). The majority of the concentrations in this group (with the exception of liver) were lower than those from Groups 1A and 1B.

Group 3

This group, which was the largest of all, contains deaths in which methadone was an incidental finding. Table 5 displays results from this group. The average methadone concentration in vitreous was 180 ng/mL (n = 16). EDDP was detected in peripheral blood at a mean concentration of 100 ng/mL (n = 6; SD = 31; range = 63-150). Methadone was detected at an average concentration of 630 ng/mL (n = 12; SD = 370;

Table 4. Concentrations of vitreous humor, peripheral blood (femoral), heart blood, brain, gastric contents (total amount), and liver for each case in Group 2. Averages, standard deviations, number of positive cases (n), and ranges are also included.

Case	VH (ng/mL)		PB(Fem) (ng/mL)		HB (ng/mL)		Brain (ng/g)		GC (mg)		Liver (ng/g)	
	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN
9					ND	35						
10 *												
38 *	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
57	ND	93	120	550	ND	440	ND	1200	0.021	0.73	400	4400
72	ND	150	ND	600	54	680	ND	1200	ND	0.71	540	2800
87	ND	80	ND	82	66	780	ND	700	0.013	1.7	780	7900
Average	**NA**	**110**	**NA**	**410**	**60**	**480**	**NA**	**1000**	**0.017**	**1.0**	**570**	**5000**
SD	**NA**	**37**	**NA**	**290**	**8.5**	**330**	**NA**	**290**	**0.0058**	**0.57**	**190**	**2600**
n	**0**	**3**	**1**	**3**	**2**	**4**	**0**	**3**	**2**	**3**	**3**	**3**
Range	**NA**	**80-150**	**NA**	**82-600**	**54-66**	**35-780**	**NA**	**700-1200**	**0.013-0.021**	**0.71-1.7**	**400-780**	**2800-7900**

* Additional specimens analyzed

Table 5. Concentrations of vitreous humor, peripheral blood (femoral), heart blood, brain, gastric contents (total amount), and liver for each case in Group 3. Averages, standard deviations, number of positive cases (n), and ranges are also included.

	VH (ng/mL)		PB(Fem) (ng/mL)		HB (ng/mL)		Brain (ng/g)		GC (mg)		Liver (ng/g)	
Case	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN
2					ND	30						
3 *												
4 *												
5					ND	71						
6 *												
12					ND	240						
13					37	350						
14					62	350						
16					100	900					700	5300
21					30	140						
26			84	840								
27 *												
28 *												
31	ND	240			110	670	ND	1700	ND	0.21	1600	6200
33 *	ND	38	ND	450	ND	650	ND	1300	ND	0.42	300	4200
37	ND	140	86	440	57	620	ND	1200	0.088	3.8	370	2300
39	ND	110	ND	430	ND	370	ND	900	ND	0.80	480	2900
45	ND	69	ND	220	71	600			ND	1.3		
48	ND	280	150	1400	180	2300			ND	0.79	1300	19000
58	ND	290	100	890	66	970	ND	2100				
60 *	ND	32										
61	ND	100			ND	450						
64	ND	120	ND	280	ND	170						
69 *	ND	160			94	690	ND	1800	ND	0.23	1100	3700
70	ND	89			ND	380						
71 *												
75	ND	100	120	860	100	800						

Table 5 (cont'd).

Case	VH (ng/mL)		PB(Fem) (ng/mL)		HB (ng/mL)		Brain (ng/g)		GC (mg)		Liver (ng/g)	
	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN
78	ND	100	ND	640	ND	600	ND	1100	ND	0.71	240	3200
81 *												
84	ND	400	63	950	52	910					340	5600
85					ND	130						
90 *	ND	670					ND	4600				
93	ND	ND	ND	120	ND	440	ND	220	0.090	4.6	1200	4200
Average	**NA**	**180**	**100**	**630**	**80**	**560**	**NA**	**1700**	**0.089**	**1.4**	**760**	**5700**
SD	**NA**	**160**	**31**	**370**	**41**	**470**	**NA**	**1200**	**0.0014**	**1.6**	**490**	**4800**
n	**0**	**16**	**6**	**12**	**12**	**23**	**0**	**10**	**2**	**9**	**10**	**10**
Range	**NA**	**32-670**	**63-150**	**120-1400**	**30-180**	**30-2300**	**NA**	**220-5600**	**0.088-0.090**	**0.21-4.6**	**240-1600**	**2300-19000**

* Additional specimens analyzed

range = 120-1400). The average EDDP amount in heart blood was 80 ng/mL (n = 12) while that for methadone was 560 ng/mL (n = 23). The average concentration of methadone detected in brain was 1700 ng/g (n = 10). For gastric, the mean total amount of methadone was 1.4 mg (n = 9), and that for EDDP was 0.089 mg (n = 2).

The average liver concentration was 760 ng/g for EDDP (n = 10; SD = 490; range = 240-1600) and 5700 ng/g for methadone (n = 10; SD = 4800; range = 2300-19000). This maximum concentration of methadone in liver (19000 ng/g) was seen with Case 48. This was the greatest liver concentration amongst all the groups. It is very interesting to note that the decedent here was an African American female (34 years old) who was pregnant and taking methadone for heroin and cocaine addiction. The cause of death in this case was ruptured ectopic pregnancy—not drug toxicity. Even though this was the maximum concentration of methadone detected in liver for this project, studies have actually shown increased clearance rates of methadone in pregnant women (particularly during the second and third trimesters) (Nanovskaya et al., 2004).

A study by Wong et al. (2003) involved a female decedent who was six months pregnant. This woman had a known history of drug and alcohol abuse and had been taking methadone and amitriptyline (an antidepressant). The methadone levels in this case were elevated due to deficient CYP2D6 metabolism, resulting from a mutation of this enzyme. Perhaps genetic predisposition causing poor drug metabolism is the explanation for the high methadone levels seen with Case 48 in this study. Another explanation could be that the woman had acquired tolerance to methadone after possible prolonged use. This case clearly illustrates the problem of overlapping concentrations in methadone-related deaths.

Group 4

Group 4 consists of natural deaths that were aggravated by methadone. Table 6 contains information on concentrations for this group. The average concentrations of EDDP and methadone in peripheral blood were 100 ng/mL (n = 4; SD = 45; range = 60-140) and 400 ng/mL (n = 7; SD = 150; range = 160-630), respectively. The mean values in heart blood were 73 ng/mL for EDDP (n = 5) and 410 ng/mL for methadone (n = 6). The average methadone concentration in vitreous was 120 ng/mL (n = 7) and in brain was 1000 ng/g (n = 6). The average total EDDP content in stomach contents was 0.13 mg (n = 3). The mean total methadone content was 1.5 mg (n = 5).

Liver EDDP concentrations ranged from 240-1000 ng/g with an average of 560 ng/g (n = 6; SD = 290). Methadone was detected at a mean concentration of 2700 ng/g (n = 6; SD = 1500; range = 1400-5600). The maximum liver concentration (Case 80) was more than double that of any other reported amount in this group. This person died from bronchopneumonia, which was aggravated by methadone. The presence of pneumonia in such cases often indicates methadone ingestion or complications of intravenous opioid use (Ropero-Miller and Winecker, 2004; Karch and Stephens, 2000).

Group 5

The final group contains the 12 cases that are either undetermined or pending and could thus not be grouped in one of the main groups. The average vitreous concentration for methadone was 87 ng/mL (n = 8). That for brain was 1100 ng/g (n = 8). Peripheral blood had a mean EDDP concentration of 91 ng/mL (n = 5) and a mean methadone concentration of 310 ng/mL (n = 9). The averages in heart blood were 110 ng/mL for EDDP (n = 4) and 300 ng/mL for methadone (n = 9). The average total amount of

Table 6. Concentrations of vitreous humor, peripheral blood (femoral), heart blood, brain, gastric contents (total amount), and liver for each case in Group 4. Averages, standard deviations, number of positive cases (n), and ranges are also included.

Case	VH (ng/mL)		PB(Fem) (ng/mL)		HB (ng/mL)		Brain (ng/g)		GC (mg)		Liver (ng/g)	
	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN
24					37	170						
40 *	ND	69	ND	160			ND	660			580	1800
42	ND	91	60	280	58	260	ND	1100	0.12	0.86	580	2300
44	ND	160	140	440	99	450	ND	1500	0.13	1.0	720	2600
52	ND	67	ND	630								
62	ND	200										
63	ND	140	ND	470	ND	450	ND	860	ND	0.55	250	2300
67	ND	110	65	400	60	560	ND	860	ND	2.3	240	1400
80			140	440	110	600	ND	1100	0.14	3.0	1000	5600
Average	**NA**	**120**	**100**	**400**	**73**	**410**	**NA**	**1000**	**0.13**	**1.5**	**560**	**2700**
SD	**NA**	**50**	**45**	**150**	**31**	**170**	**NA**	**290**	**0.0092**	**1.0**	**290**	**1500**
n	**0**	**7**	**4**	**7**	**5**	**6**	**0**	**6**	**3**	**5**	**6**	**6**
Range	**NA**	**67-200**	**60-140**	**160-630**	**37-110**	**170-600**	**NA**	**660-1500**	**0.12-0.14**	**0.55-3.0**	**240-1000**	**1400-5600**

* Additional specimens analyzed

methadone in the gastric contents was 0.71 mg (n = 6), which was the smallest average seen for all the groups. The mean liver concentration was 670 ng/g for EDDP (n = 6) and 2900 ng/g for methadone (n = 9). The decedent with the highest concentration of 6800 ng/g (Case 99) had an extensive history of prescription medication abuse, which is a common finding among methadone users. All of this information for Group 5 can be found on the following page in Table 7.

Additional Specimens

As Table 8 shows, additional specimens were available for approximately a quarter of the cases. Such samples included peripheral blood (subclavian), kidney, antemortem blood and serum, blood (type not indicated), aorta blood, and cavity fluid. At the Delaware OCME, subclavian blood is typically collected during inspections when a full autopsy is not performed (whereas heart blood is collected during autopsies). Of particular interest to this project are concentrations in kidney since this is yet another type of tissue. Unfortunately though, this specimen was only available for five cases.

For Case 33, the concentration of methadone in kidney was 1100 ng/g. For this same case, methadone was found at 1300 ng/g in brain and 4200 ng/g in liver. Similarly, for Case 95, the kidney concentration for methadone was 1300 ng/g, which closely compares to the amount of 1400 ng/g in brain. The concentration in liver was 5500 ng/g. Case 99 also had comparable findings. The amount of methadone in kidney was 2000 ng/g. That in brain was 1900 ng/g, while that in liver was 6800 ng/g. These three specific cases suggest that methadone concentrations are similar in brain and kidney. Liver, on the other hand, tends to have the highest concentration of all the samples.

Table 7. Concentrations of vitreous humor, peripheral blood (femoral), heart blood, brain, gastric contents (total amount), and liver for each case in Group 5. Averages, standard deviations, number of positive cases (n), and ranges are also included.

Case	VH (ng/mL)		PB(Fem) (ng/mL)		HB (ng/mL)		Brain (ng/g)		GC (mg)		Liver (ng/g)	
	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN
11			120	390								
47					ND	150	ND	1300			ND	2600
79	ND	44	ND	230	ND	150	ND	480	ND	0.80	470	1300
88 *							ND	1000	ND	0.12	180	3700
92			60	110	89	77					400	470
94	ND	45	ND	220	ND	350	ND	470	ND	0.27	ND	1400
95 *	ND	100	93	390	130	410	ND	1400	0.18	1.2	1100	5500
96 *	ND	58	ND	320								
97	ND	130	63	580	97	570	ND	1500	ND	1.5	390	2600
98	ND	73	ND	210	ND	250	ND	680	ND	0.34	ND	1700
99 *	ND	200	120	350	140	570	ND	1900			1500	6800
100	ND	44			ND	170						
Average	**NA**	**87**	**91**	**310**	**110**	**300**	**NA**	**1100**	**NA**	**0.71**	**670**	**2900**
SD	**NA**	**55**	**29**	**140**	**25**	**180**	**NA**	**520**	**NA**	**0.57**	**510**	**2100**
n	**0**	**8**	**5**	**9**	**4**	**9**	**0**	**8**	**1**	**6**	**6**	**9**
Range	**NA**	**44-200**	**60-120**	**110-580**	**89-140**	**77-570**	**NA**	**470-1900**	**NA**	**0.12-1.5**	**180-1500**	**470-6800**

* Additional specimens analyzed

Table 8. Concentrations of additional specimens for each case in all six groups.

Group	Case	PB(Sub) (ng/mL)		Kidney (ng/g)		Ante Blood (ng/mL)		Ante Serum (ng/mL)		Blood (ng/mL)		Aorta Bld (ng/mL)		Cavity Fluid (ng/mL)	
		EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN
1A	36	90	1500												
1B	8					280	400								
	18											180	1300		
	23					ND	33								
	54	ND	230												
	55	ND	180												
	56	ND	360												
	77	110	760												
2	10									ND	230				
	38			ND	ND										
3	3									210	790				
	4													ND	890
	6									74	730				
	27	61	330												
	28	56	270												
	33			1000	1100										
	60	ND	150												
	69									ND	530				
	71					150	260	ND	60						
	81					ND	130	ND	ND						
	90	100	2900												
4	40	ND	180												
5	88			830	600										
	95			4800	1300										
	96	ND	250												
	99			900	2000	82	270	67	210						

Summary

Table 9 is included to summarize all the averages, standard deviations, and ranges for each of the specimens in each of the groups. Additionally, bar graphs have been incorporated to show the average concentrations of the main specimen types in each of the six groups (Figures 8-13). Both this table and these figures illustrate that the average methadone concentration was highest in Group 1A for all specimens except liver. Group 3 had the greatest concentration, and this is also the group that contained the value of 19000 ng/g (the pregnant woman). Even after eliminating this value from the average, it is still 4200 ng/g, which would be the second highest average after Group 2. Even with all the variables affecting methadone concentrations and metabolism, one would expect to see the greatest concentrations of methadone in each of the samples in Group 1A since this is the group in which death was due solely to methadone toxicity.

While viewing the bar graph for vitreous humor, one can see that EDDP is not readily detected in this matrix. Ziminski et al. (1984) studied methadone, barbiturates, and morphine in vitreous humor, blood, and several tissues. Their study showed that water-soluble drugs are more likely to diffuse from the blood to the vitreous. Drugs must also have adequate lipid solubility and must not be significantly affected by protein binding. This suggests that EDDP does not have these characteristics since it was only detected in two cases. The group with the second highest methadone average in vitreous was Group 3—the group in which methadone was an incidental finding.

Figure 9 graphically shows the results for femoral blood. As was already mentioned, the average is greatest for Group 1A. When comparing the bar graphs for all the blood and tissue samples, it is clear that the average for femoral blood stands out most

Table 9. Table summarizing the averages, standard deviations, number of positive cases (n), and ranges for listed specimens in each group.

Group		VH (ng/mL)		PB(Fem) (ng/mL)		HB (ng/mL)		Brain (ng/g)		GC (mg)		Liver (ng/g)	
		EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN	EDDP	MDN
1A	**Average**	**NA**	**260**	**110**	**950**	**96**	**590**	**NA**	**1800**	**0.11**	**2.3**	**680**	**4000**
	SD	NA	270	54	510	19	390	NA	960	0.069	2.6	250	1400
	n	1	10	8	9	6	12	0	8	3	7	7	8
	Range	NA	60-840	50-220	330-1800	77-130	130-1500	NA	580-3500	0.045-0.18	0.34-7.2	310-970	1500-5600
1B	**Average**	**NA**	**160**	**180**	**640**	**190**	**530**	**NA**	**1300**	**NA**	**0.80**	**530**	**3600**
	SD	NA	130	130	370	250	380	NA	660	NA	1.5	300	2000
	n	1	16	10	16	11	20	0	13	1	14	15	16
	Range	NA	47-470	64-450	170-1500	32-900	92-1400	NA	490-2900	NA	0.010-5.9	220-1500	990-9200
2	**Average**	**NA**	**110**	**NA**	**410**	**60**	**480**	**NA**	**1000**	**0.017**	**1.0**	**570**	**5000**
	SD	NA	37	NA	290	8	330	NA	290	0.0058	0.57	190	2600
	n	0	3	1	3	2	4	0	3	2	3	3	3
	Range	NA	80-150	NA	82-600	54-66	35-780	NA	700-1200	0.013-0.021	0.71-1.7	400-780	2800-7900
3	**Average**	**NA**	**180**	**100**	**630**	**80**	**560**	**NA**	**1700**	**0.089**	**1.4**	**760**	**5700**
	SD	NA	160	31	370	41	470	NA	1200	0.0014	1.6	490	4800
	n	0	16	6	12	12	23	0	10	2	9	10	10
	Range	NA	32-670	63-150	120-1400	30-180	30-2300	NA	220-5600	0.088-0.090	0.21-4.6	240-1600	2300-19000
4	**Average**	**NA**	**120**	**100**	**400**	**73**	**410**	**NA**	**1000**	**0.13**	**1.5**	**560**	**2700**
	SD	NA	50	45	150	31	170	NA	290	0.0092	1.0	290	1500
	n	0	7	4	7	5	6	0	6	3	5	6	6
	Range	NA	67-200	60-140	160-630	37-110	170-600	NA	660-1500	0.12-0.14	0.55-3.0	240-1000	1400-5600
5	**Average**	**NA**	**87**	**91**	**310**	**110**	**300**	**NA**	**1100**	**NA**	**0.71**	**670**	**2900**
	SD	NA	55	29	140	25	180	NA	520	NA	0.57	510	2100
	n	0	8	5	9	4	9	0	8	1	6	6	9
	Range	NA	44-200	60-120	110-580	89-140	77-570	NA	470-1900	NA	0.12-1.5	180-1500	470-6800

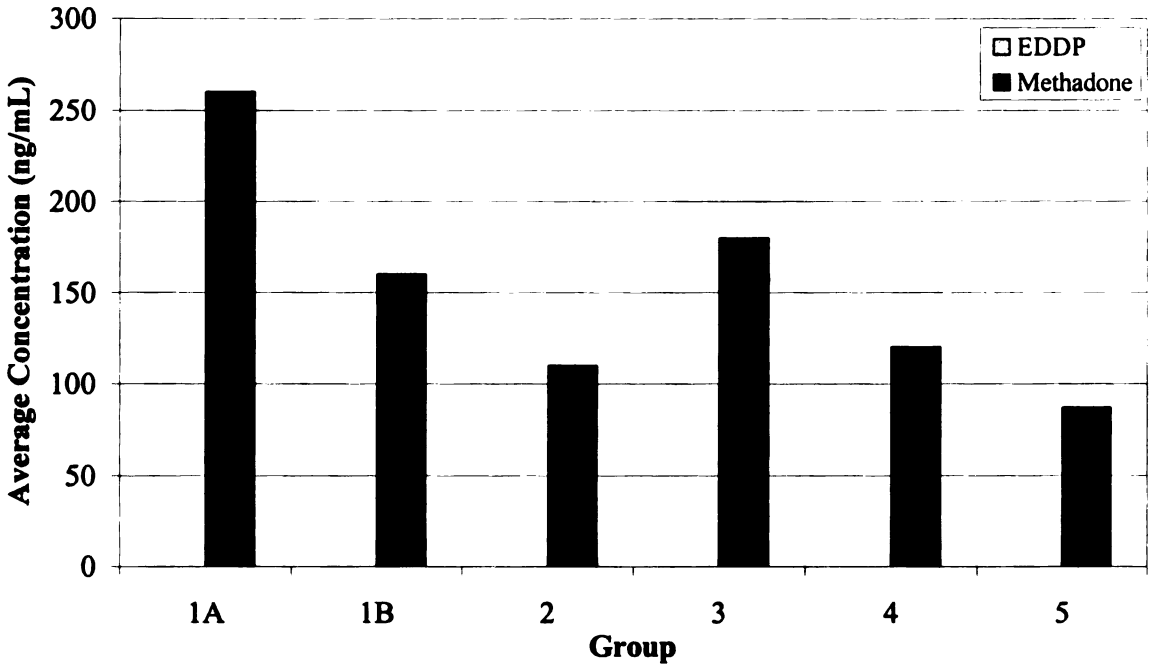

Figure 8. Bar graph showing the average concentrations in ng/mL of EDDP and methadone in vitreous humor for the six groups.

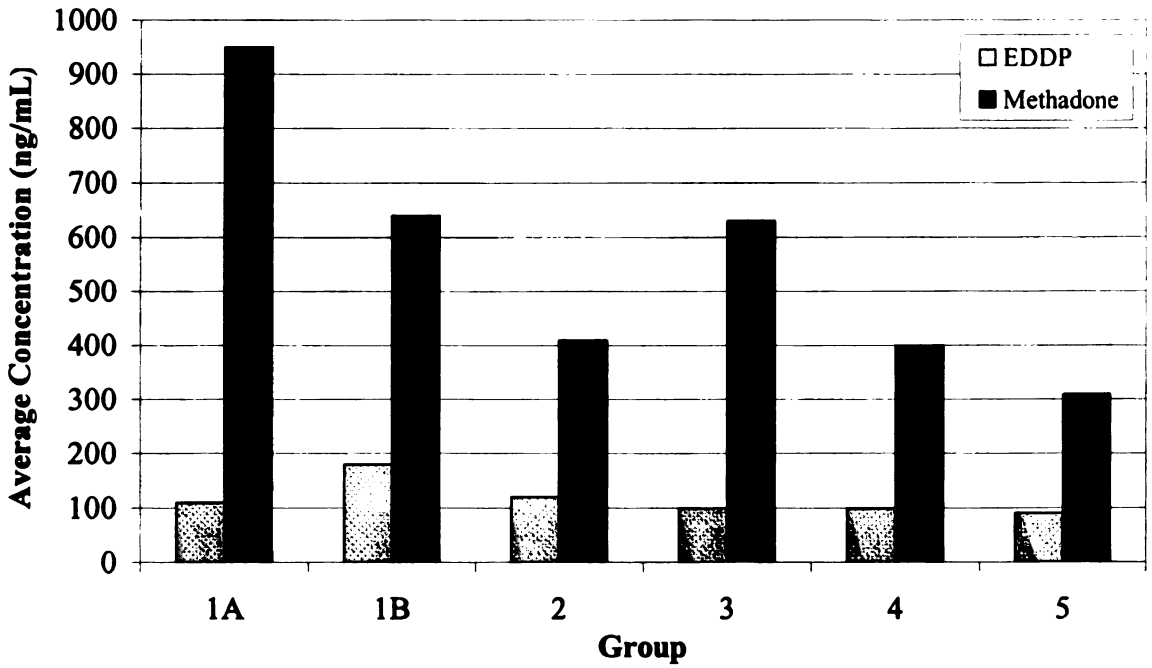

Figure 9. Bar graph showing the average concentrations in ng/mL of EDDP and methadone in peripheral blood (femoral) for the six groups.

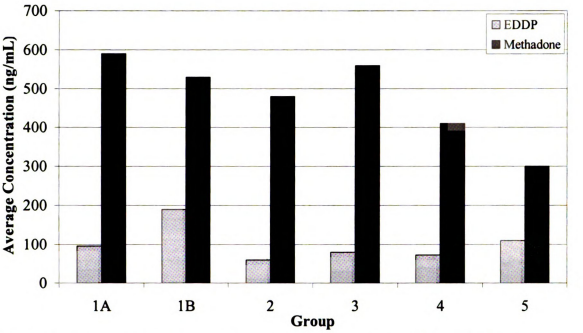

Figure 10. Bar graph showing the average concentrations in ng/mL of EDDP and methadone in heart blood for the six groups.

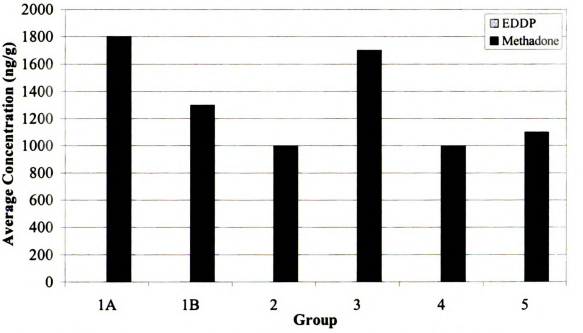

Figure 11. Bar graph showing the average concentrations in ng/g of EDDP and methadone in brain for the six groups.

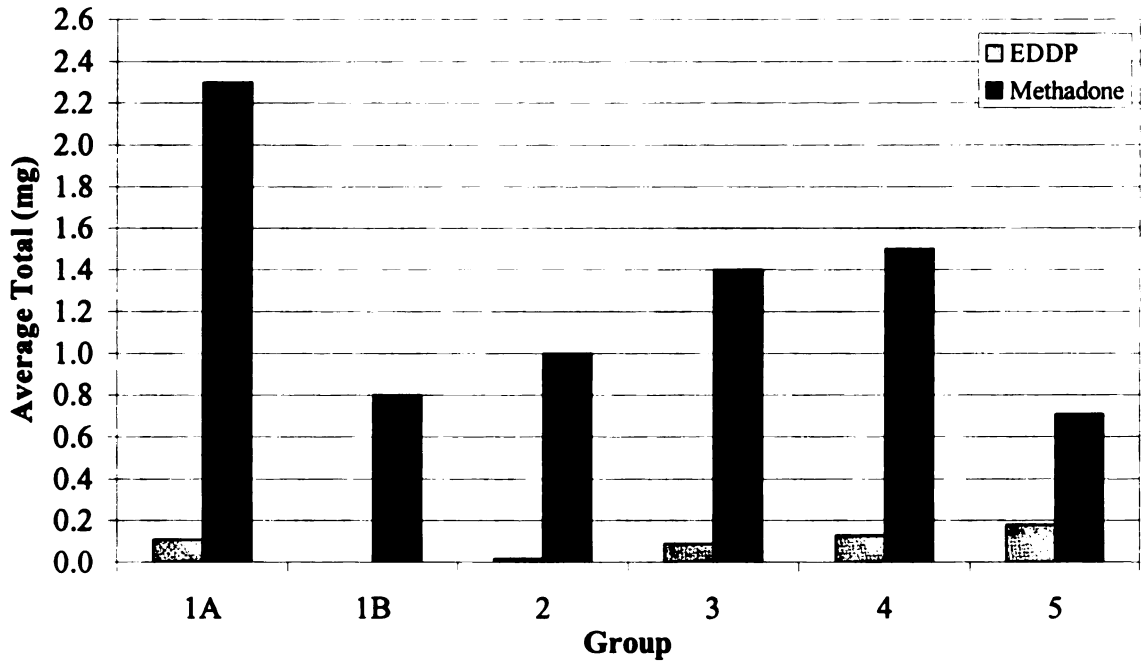

Figure 12. Bar graph showing the average totals in mg of EDDP and methadone in gastric contents for the six groups.

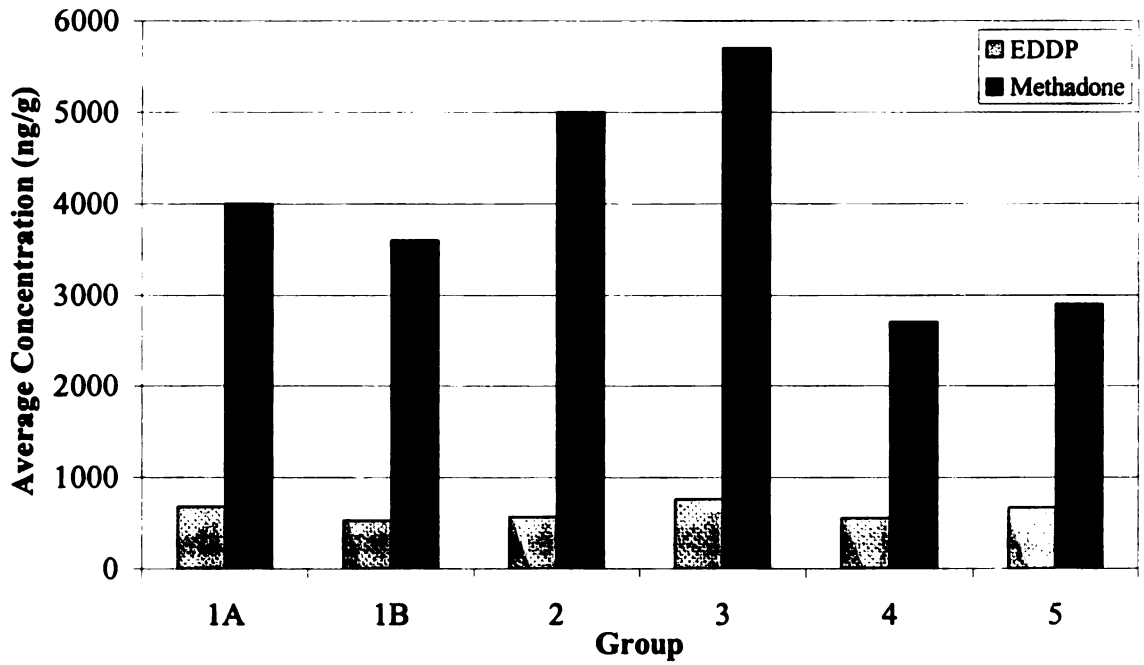

Figure 13. Bar graph showing the average concentrations in ng/g of EDDP and methadone in liver for the six groups.

as being the greatest. For the other sample types, the averages are closer in range, so the bar lines are closer in height. For peripheral blood, the group with the second highest average was 1B (multiple drug deaths involving methadone), which again would be expected. Group 3 was very close though; there was only a 10 ng/mL difference between Groups 1B and 3. Methadone's metabolite had the highest average in Group 1B, and all the others were very similar in concentration.

The heart blood graph (Figure 10) reveals that concentrations in this specimen are close in range amongst the various groups (mainly Groups 1A, 1B, 2, and 3). Group 1B has the largest EDDP concentration again. Brain is similar to vitreous in that EDDP is not normally present. Figure 11 illustrates that Group 1A has the greatest concentration of methadone in brain, followed by Group 3. The figure for gastric contents is interesting as well. The average total for Group 1A is far greater than those for the other groups. Again, this would be expected since these are deaths of known methadone toxicity. Surprisingly though, all groups registered having EDDP except Group 1B. This is probably just coincidental since this compound is not readily present in gastric contents.

The bar graph for liver concentrations is the most baffling of the six. The reason for this is that Group 3 has the maximum average followed by Group 2, and that is not what would be expected. It is rather difficult to assess these results without a more detailed history for each case including how long each person had been on methadone. This information would help determine a person's potential tolerance level at the time of death. People who are poor metabolizers would have increased concentrations of methadone. From the provided histories, it is hard to determine which individuals, if any, had slower metabolisms. Thus, no additional conclusions could be made from this data.

Looking back to the cases with kidney samples, one can see that the concentration of methadone in liver is between three and four times the concentration in brain or kidney. This data compares closely to published data containing methadone concentrations in brain and liver. It is important to note though that such data is scarce. In an article by Bastos and Galante (1976) on traumatic deaths, they report median blood and brain concentrations of 0.13 mg/100 mL. A median liver concentration of 0.53 mg/100 mL is also given. From these averages, one can see that the median liver concentration is four times that of brain.

Ratios of Methadone to EDDP and Other Matrices to Blood

Most published data on methadone-related deaths focus on determining a lethal concentration range. There are also many articles devoted to the pharmacodynamics and pharmacokinetics of this drug. The specimen commonly analyzed in these studies is blood (most often peripheral). A purpose of this study was to determine if other sample types provide valuable information for the interpretation of methadone deaths. Another focus was to study whether or not ratios of parent drug to metabolite and ratios of other matrices to peripheral blood would help in classifying methadone deaths. When possible, ratios of methadone to EDDP were calculated for peripheral blood (femoral), heart blood, and liver. Additionally, ratios of methadone in vitreous, brain, and liver to methadone in femoral blood were also calculated. These results are displayed in tables for each of the groups. Averages, standard deviations, and ranges are included.

Group 1A

For methadone intoxication deaths (Table 10), the average methadone-to-EDDP ratio was 11 for peripheral blood (n = 8), 8.9 for heart blood (n = 6), and 7.0 for liver (n = 7). The average ratio of vitreous to peripheral blood for methadone was 0.27 (n = 9). The corresponding ratio for brain was 2.2 (n = 7), and that for liver was 5.7 (n = 7). Ropero-Miller and Winecker (2004) report liver-to-central ratios of 6.2 and 7.5 for two different groups of methadone-related deaths. These are in the same broad range as the average for this study. Perhaps the reason for their increased ratio is that they used central blood for the calculation rather than peripheral blood. Another explanation could be a different sampling site in the liver.

The maximum ratio of methadone to EDDP in peripheral blood was 24 and in liver was 10. These were seen with Case 74. The maximum in heart blood was 18 (Case 68). Both of these high ratios can be partially explained by information obtained from the investigative reports. Case 74 involved a 23-year-old Caucasian male who recently purchased methadone tablets from an illicit source. If this was one of his initial exposures to methadone, then it would likely result in acute overdose. The second case was a suicide, so intentional overdose would justify the high ratio.

Group 1B

Table 11 contains ratios for Group 1B. The mean methadone-to-metabolite ratio was 5.1 for peripheral blood (n = 10), 6.1 for heart blood (n = 11), and 7.7 for liver (n = 15). Case 7 had the highest ratio in heart blood, and perhaps this was due to a recent switch from a morphine-based medication to methadone. Methadone is not cross-tolerant to morphine tolerance, which could help explain the ratio (Garrido and Trocóniz, 1999).

Table 10. Ratios for cases in Group 1A. Averages, standard deviations, number of cases (n), and ranges are also included.

Case	MDN/EDDP Ratio for PB(Fem)	MDN/EDDP Ratio for HB	MDN/EDDP Ratio for Liver	Vitreous/PB(Fem) Ratio for MDN	Brain/PB(Fem) Ratio for MDN	Liver/PB(Fem) Ratio for MDN
36	12			0.49		
50	5.0		5.2	0.24	2.8	11
59		7.4	5.9			
65	9.5	7.4	6.7	0.25	2.1	7.9
66				0.18	1.8	4.5
68	16	18	5.3	0.17	1.5	2.7
74	24		10	0.10	1.8	3.1
76	7.9	7.7	7.1	0.15		5.1
82	5.5	6.7		0.70	2.9	
89	6.0	6.3	8.4	0.19	2.3	5.9
Average	**11**	**8.9**	**7.0**	**0.27**	**2.2**	**5.7**
SD	**6.6**	**4.3**	**1.8**	**0.19**	**0.55**	**2.9**
n	**8**	**6**	**7**	**9**	**7**	**7**
Range	**5.0-24**	**6.3-18**	**5.2-10**	**0.10-0.70**	**1.5-2.9**	**2.7-11**

Table 11. Ratios for cases in Group 1B. Averages, standard deviations, number of cases (n), and ranges are also included.

Case	MDN/EDDP Ratio for PB(Fem)	MDN/EDDP Ratio for HB	MDN/EDDP Ratio for Liver	Vitreous/PB(Fem) Ratio for MDN	Brain/PB(Fem) Ratio for MDN	Liver/PB(Fem) Ratio for MDN
7		16				
17		4.7				
19	6.1	4.3				
22		2.3				
29	6.1					
30				0.20	2.2	4.3
32			12	0.13	2.2	7.6
34	9.4	8.8	9.8	0.43	4.2	8.4
35			3.0			
41	5.5	6.9	5.0	0.22		5.6
43			8.1	0.22	2.0	7.4
46			8.8			
49	5.4	4.8		0.27		
51			11	0.31	3.1	10
53		1.2	7.4	0.17	1.9	5.6
54			8.8			
55			13			
56			5.8			
73	1.2		7.9	0.16	1.4	11
77	0.38			0.65		
83	9.8	9.1	6.6		3.0	6.0
86	4.3	3.5	2.3	0.35	3.6	4.3
91	3.3	5.6	6.1	0.53	2.1	10
Average	**5.1**	**6.1**	**7.7**	**0.30**	**2.6**	**7.3**
SD	**3.1**	**4.1**	**3.1**	**0.16**	**0.87**	**2.3**
n	**10**	**11**	**15**	**12**	**10**	**11**
Range	**0.38-9.8**	**1.2-16**	**2.3-13**	**0.10-0.65**	**1.4-4.2**	**4.3-11**

The average ratio of vitreous to femoral blood was 0.30 (n = 12). The average ratio of brain to femoral blood was 2.6 (n = 10), and that for liver was 7.3 (n = 11).

Group 2

Ratios for Group 2 are listed in Table 12. The mean methadone-to-EDDP ratio was 12 for heart blood (n = 2), and 8.8 for liver (n = 3). The average ratio of vitreous to peripheral blood was 0.46 (n = 3), that for brain was 4.2 (n = 3), and that for liver was 36 (n = 3). The reason for this extremely high liver-to-blood ratio is due to Case 87, which has an individual ratio of 96. This decedent was in early stages of decomposition, which could have affected the concentrations. This case is one that demonstrates why it is important to know the history and circumstances surrounding death before interpreting the drug findings.

Group 3

Table 13 consists of information for Group 3. In peripheral blood, the average ratio of methadone to EDDP was 9.3 (n = 6). The ratio in heart blood was 9.5 (n = 12) and in liver was 8.9 (n = 10). All ratios are fairly close in range here. Moreover, the maximum in each of these three groups was seen with Case 84—a Caucasian male (44 years old) with a history of asthma, Hepatitis C, and heavy smoking. The average ratio of vitreous to peripheral blood was 0.26 (n = 10). The brain-to-blood ratio was 2.3 (n = 6), and the liver-to-blood ratio was 12 (n = 7).

Group 4

The average ratio of methadone to EDDP in peripheral blood for Group 4 was 4.3 (n = 4). This data can be found in Table 14. The ratio in heart blood was 5.7 (n = 4) and in liver was 5.2 (n = 6). The mean ratio of vitreous to peripheral blood was 0.30 (n = 6).

Table 12. Ratios for cases in Group 2. Averages, standard deviations, number of cases (n), and ranges are also included.

Case	MDN/EDDP Ratio for PB(Fem)	MDN/EDDP Ratio for HB	MDN/EDDP Ratio for Liver	Vitreous/PB(Fem) Ratio for MDN	Brain/PB(Fem) Ratio for MDN	Liver/PB(Fem) Ratio for MDN
57	4.6		11	0.17	2.2	8.0
72		13	5.2	0.25	2.0	4.7
87		12	10	1.0	8.5	96
Average	**NA**	**12**	**8.8**	**0.46**	**4.2**	**36**
SD	**NA**	**0.5**	**3.1**	**0.44**	**3.7**	**52**
n	**1**	**2**	**3**	**3**	**3**	**3**
Range	**NA**	**12-13**	**5.2-11**	**0.17-1.0**	**2.0-8.5**	**4.7-96**

Table 13. Ratios for cases in Group 3. Averages, standard deviations, number of cases (n), and ranges are also included.

Case	MDN/EDDP Ratio for PB(Fem)	MDN/EDDP Ratio for HB	MDN/EDDP Ratio for Liver	Vitreous/PB(Fem) Ratio for MDN	Brain/PB(Fem) Ratio for MDN	Liver/PB(Fem) Ratio for MDN
13		9.5				
14		5.6				
16		9.0	7.6			
21		4.7				
26	10					
31		6.1	3.9			
33			14	0.084	2.9	9.3
37	5.1	11	6.2	0.32	2.7	5.2
39			6.0	0.26	2.1	6.7
45		8.5		0.31		
48	9.3	13	15	0.20		14
58	8.9	15		0.33	2.4	
64				0.43		
69		7.3	3.4			
75	7.2	8.0		0.12		
78			13	0.16	1.7	5.0
84	15	18	16	0.42		5.9
93			3.5		1.8	35
Average	**9.3**	**9.5**	**8.9**	**0.26**	**2.3**	**12**
SD	**3.3**	**3.8**	**5.1**	**0.12**	**0.5**	**11**
n	**6**	**12**	**10**	**10**	**6**	**7**
Range	**5.1-15**	**4.7-18**	**3.4-16**	**0.084-0.43**	**1.7-2.9**	**5.0-35**

Table 14. Ratios for cases in Group 4. Averages, standard deviations, number of cases (n), and ranges are also included.

Case	MDN/EDDP Ratio for PB(Fem)	MDN/EDDP Ratio for HB	MDN/EDDP Ratio for Liver	Vitreous/PB(Fem) Ratio for MDN	Brain/PB(Fem) Ratio for MDN	Liver/PB(Fem) Ratio for MDN
24		4.6				
40			3.1	0.43	4.1	11
42	4.7	4.5	4.0	0.33	3.9	8.2
44	3.1	4.5	3.6	0.36	3.4	5.9
52				0.11		
63			9.2	0.30	1.8	4.9
67	6.2	9.3	5.8	0.28	2.2	3.5
80	3.1		5.6		2.5	13
Average	**4.3**	**5.7**	**5.2**	**0.30**	**3.0**	**7.7**
SD	**1.4**	**2.4**	**2.2**	**0.11**	**1.0**	**3.7**
n	**4**	**4**	**6**	**6**	**6**	**6**
Range	**3.1-6.2**	**4.5-9.3**	**3.1-9.2**	**0.11-0.43**	**1.8-4.1**	**3.5-13**

That for brain was 3.0 (n = 6), and that for liver was 7.7 (n = 6). The maximum ratio of liver to blood in this group was 13 (Case 80). This was the person who died from bronchopneumonia (aggravated by drugs).

Group 5

The various ratios were determined for Group 5 as well. The average ratio of parent drug to metabolite was 4.3 for peripheral blood (n = 5), 3.5 for heart blood (n = 4), and 6.8 for liver (n = 6). It is interesting to note that the maximum ratio in liver was from Case 88, which was an exhumed body. The body was exhumed due to suspicion of poisoning, which surfaced after burial. The mean vitreous-to-blood ratio was 0.28 (n = 7). The average ratio of brain to blood was 3.2 (n = 6), and the average ratio of liver to blood was 8.9 (n = 7). This information is listed in Table 15.

Additional Specimens

When possible, ratios were determined for additional specimens as well (Table 16). The ratio of methadone to EDDP was calculated for peripheral blood (subclavian), kidney, antemortem blood and serum, blood, and aorta blood. These ratios were low for kidney and antemortem blood and serum (compared to those seen with the typical specimens). Additionally, subclavian-to-femoral ratios were determined for methadone as well as kidney-to-blood ratios.

In this study, antemortem blood was only available in five cases. Antemortem serum was also available in three of these cases. The work of Cook et al. (2000) showed that postmortem-to-antemortem (PM/AM) ratios are typically similar to central-to-peripheral (C/P) ratios. When the C/P ratio is high for a given drug, it is likely that the PM/AM ratio will also be high. In 11 cases they examined, the average PM/AM ratio

Table 15. Ratios for cases in Group 5. Averages, standard deviations, number of cases (n), and ranges are also included.

Case	MDN/EDDP Ratio for PB(Fem)	MDN/EDDP Ratio for HB	MDN/EDDP Ratio for Liver	Vitreous/PB(Fem) Ratio for MDN	Brain/PB(Fem) Ratio for MDN	Liver/PB(Fem) Ratio for MDN
11	3.3					
79			2.8	0.19	2.1	5.7
88			21			
92	1.8	0.87	1.2			4.3
94				0.20	2.1	6.4
95	4.2	3.2	5.0	0.26	3.6	14
96				0.18		
97	9.2	5.9	6.7	0.22	2.6	4.5
98				0.35	3.2	8.1
99	2.9	4.1	4.5	0.57	5.4	19
Average	**4.3**	**3.5**	**6.8**	**0.28**	**3.2**	**8.9**
SD	**2.9**	**2.1**	**7.0**	**0.14**	**1.3**	**5.7**
n	**5**	**4**	**6**	**7**	**6**	**7**
Range	**1.8-9.2**	**0.87-5.9**	**1.2-21**	**0.18-0.57**	**2.1-5.4**	**4.3-19**

Table 16. Ratios for additional specimens.

Group	Case	MDN/EDDP Ratio for PB(Sub)	MDN/EDDP Ratio for Kidney	MDN/EDDP Ratio for Ante Bld	MDN/EDDP Ratio for Ante Serum	MDN/EDDP Ratio for Blood	MDN/EDDP Ratio for Aorta Bld	PB(Sub)/PB(Fem) Ratio for MDN	Kidney/PB(Fem) Ratio for MDN
1A	36	17						1.1	
1B	8			1.4					
	18						7.2		
	77	6.9							
3	3					3.8			
	6					9.9			
	27	5.4							
	28	4.8							
	33		1.1						2.4
	71			1.7					
	90	29							
4	40							1.1	
5	88		0.72						
	95		0.27						3.3
	96							0.78	
	99		2.2	3.3	3.1				5.7

was 1.4. In this present study, Case 99 was the only case in which a PM/AM ratio could be calculated. The PM/AM ratio for this case was 1.3, which compares closely with the average reported by Cook et al. The C/P ratio for Case 99 was 1.6, showing that the PM/AM ratio is also similar to this value.

Summary

Table 17 is provided to summarize all the average ratios for all the different specimens and groups. Bar graphs have been included as graphical representations of this data as well (Figures 14-19). Figure 14 shows that the mean methadone-to-EDDP ratio was the greatest in Group 1A, which is again what was expected. Group 3 had the second highest ratio. In heart blood, however, the maximum ratio was seen in Group 2. This confirms that peripheral blood is the preferred specimen for analysis.

The ratios of parent drug to metabolite in liver were very similar amongst the six groups. For the ratios of alternate matrices to blood, Group 2 had the maximum average ratio for each. This is, however, due to the fact that Case 87 was in this group. Since there were only three cases, this one case significantly skewed the ratios. If this ratio of 96 for liver-to-blood (Case 87) was eliminated from the average, the average ratio would drop from 36 to 6.3. This value would place Group 2 in the same range as the other groups.

Table 17. Table summarizing the averages, standard deviations, number of cases (n), and ranges for listed ratios in each group.

Group		MDN/EDDP Ratio for PB(Fem)	MDN/EDDP Ratio for HB	MDN/EDDP Ratio for Liver	Vitreous/PB(Fem) Ratio for MDN	Brain/PB(Fem) Ratio for MDN	Liver/PB(Fem) Ratio for MDN
1A	**Average**	**11**	**8.9**	**7.0**	**0.27**	**2.2**	**5.7**
	SD	6.6	4.3	1.8	0.19	0.55	2.9
	n	8	6	7	9	7	7
	Range	5.0-24	6.3-18	5.2-10	0.10-0.70	1.5-2.9	2.7-11
1B	**Average**	**5.1**	**6.1**	**7.7**	**0.30**	**2.6**	**7.3**
	SD	3.1	4.1	3.1	0.16	0.87	2.3
	n	10	11	15	12	10	11
	Range	0.38-9.8	1.2-16	2.3-13	0.10-0.65	1.4-4.2	4.3-11
2	**Average**	**NA**	**12**	**8.8**	**0.46**	**4.2**	**36**
	SD	NA	0.55	3.1	0.44	3.7	52
	n	1	2	3	3	3	3
	Range	NA	12-13	5.2-11	0.17-1.0	2.0-8.5	4.7-96
3	**Average**	**9.3**	**9.5**	**8.9**	**0.26**	**2.3**	**12**
	SD	3.3	3.8	5.1	0.12	0.47	11
	n	6	12	10	10	6	7
	Range	5.1-15	4.7-18	3.4-16	0.084-0.43	1.7-2.9	5.0-35
4	**Average**	**4.3**	**5.7**	**5.2**	**0.30**	**3.0**	**7.7**
	SD	1.4	2.4	2.2	0.11	1.0	3.7
	n	4	4	6	6	6	6
	Range	3.1-6.2	4.5-9.3	3.1-9.2	0.11-0.43	1.8-4.1	3.5-13
5	**Average**	**4.3**	**3.5**	**6.8**	**0.28**	**3.2**	**8.9**
	SD	2.9	2.1	7.0	0.14	1.3	5.7
	n	5	4	6	7	6	7
	Range	1.8-9.2	0.87-5.9	1.2-21	0.18-0.57	2.1-5.4	4.3-19

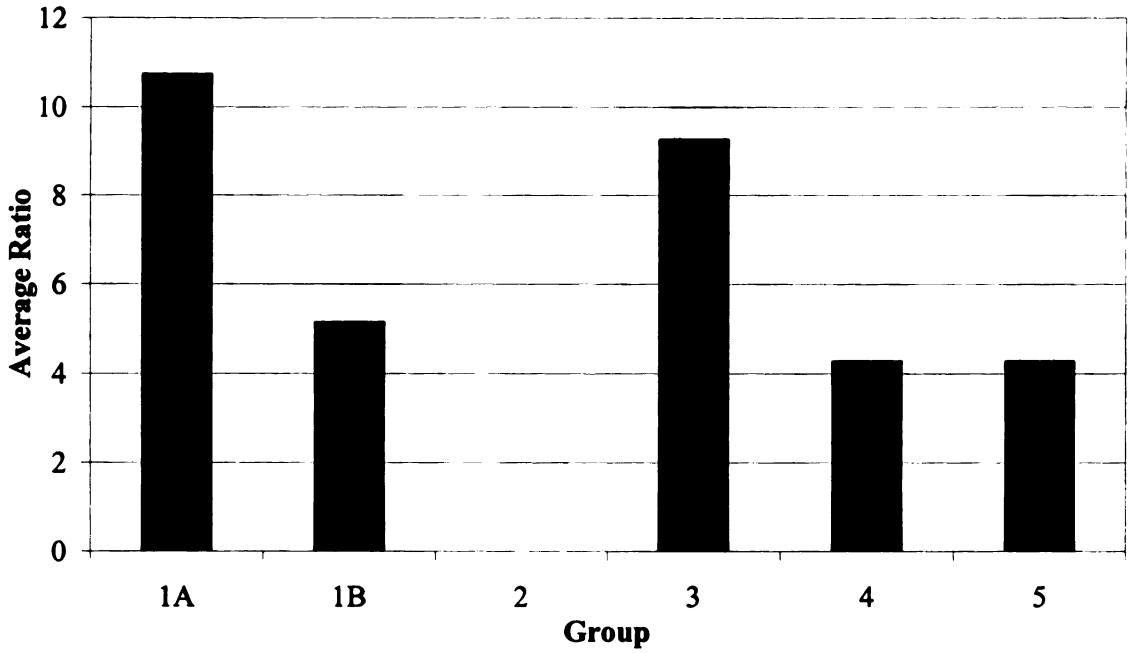

Figure 14. Bar graph showing the average ratios of methadone to EDDP in peripheral blood (femoral) for the six groups.

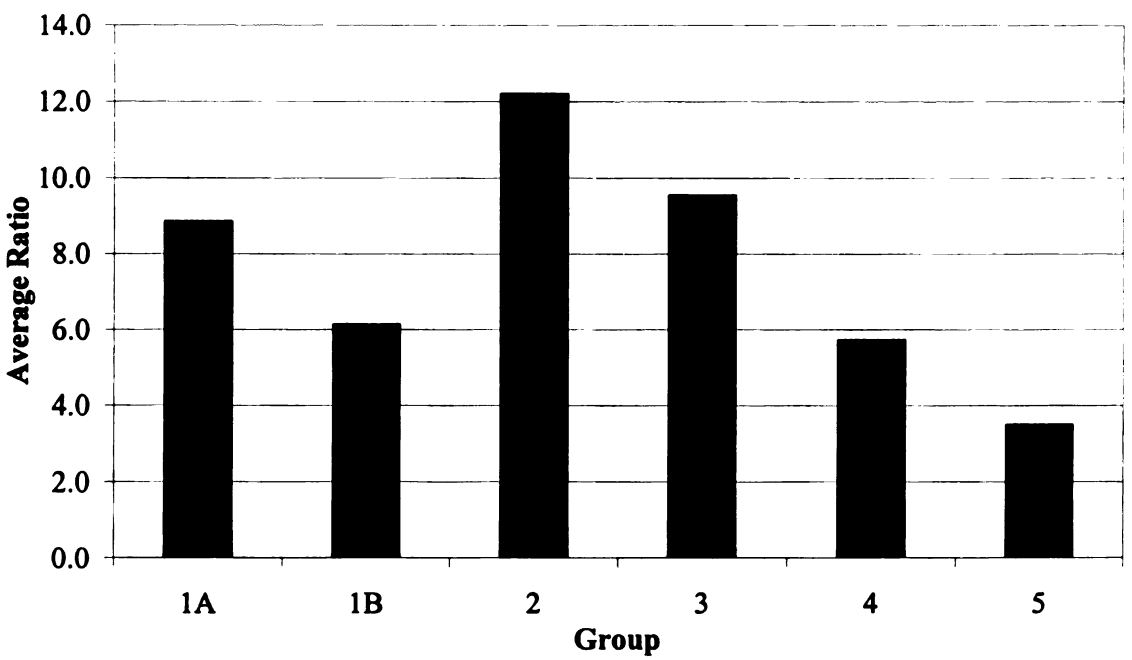

Figure 15. Bar graph showing the average ratios of methadone to EDDP in heart blood for the six groups.

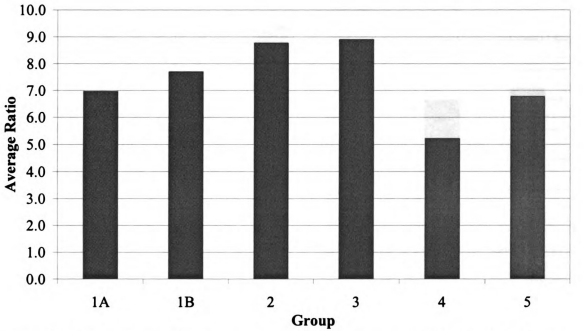

Figure 16. Bar graph showing the average ratios of methadone to EDDP in liver for the six groups.

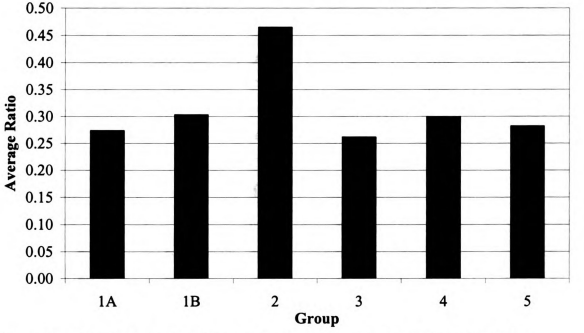

Figure 17. Bar graph showing the average ratios of methadone in vitreous humor to methadone in peripheral blood (femoral) for the six groups.

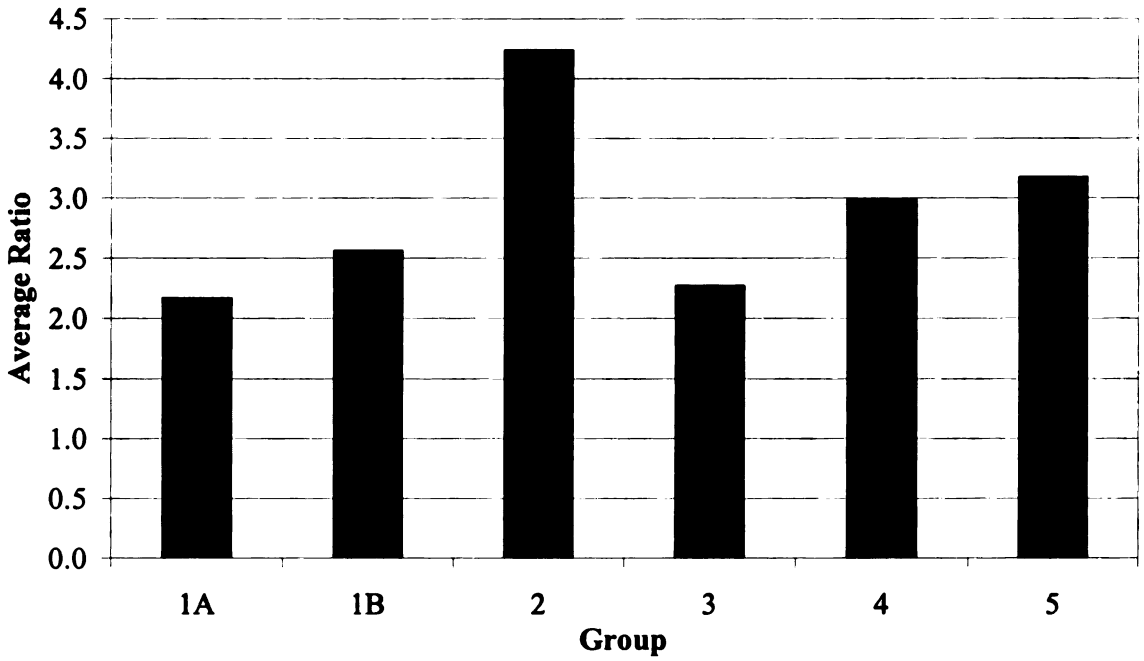

Figure 18. Bar graph showing the average ratios of methadone in brain to methadone in peripheral blood (femoral) for the six groups.

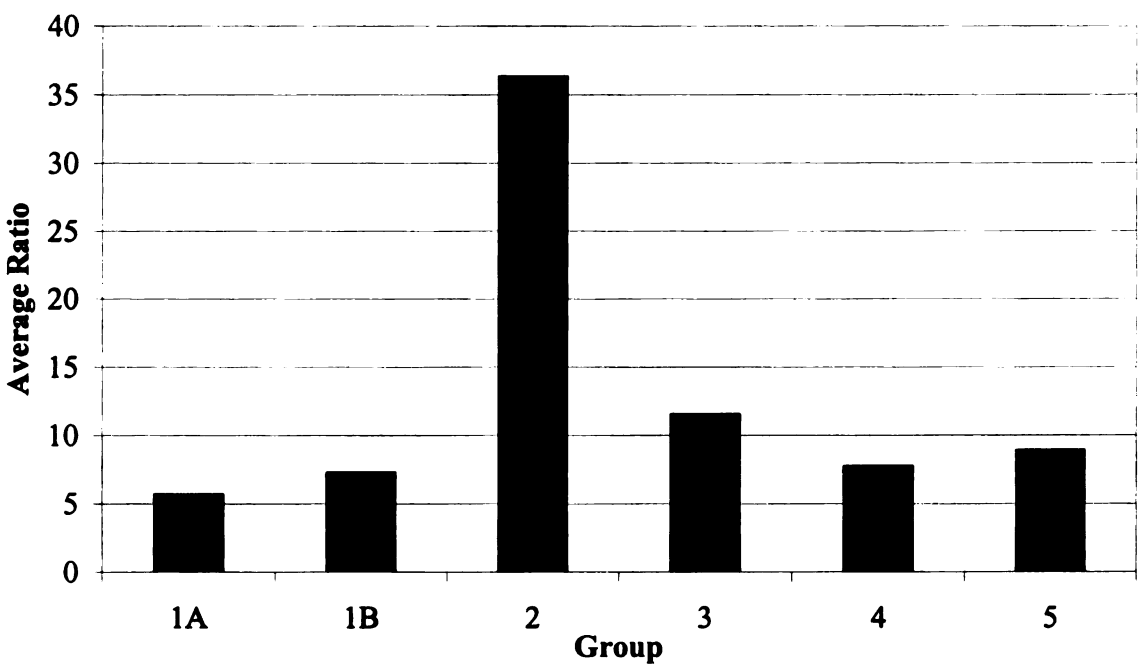

Figure 19. Bar graph showing the average ratios of methadone in liver to methadone in peripheral blood (femoral) for the six groups.

Central-to-Peripheral Ratios

Since postmortem redistribution is a recognized phenomenon with methadone, its role was considered in this project. The central blood in this study was heart blood, and femoral blood was used for the peripheral blood. The ratio of heart blood to peripheral blood was calculated for each case when possible. An average of all the cases as one big group was also determined (Table 18). The average was 1.3 with a range of 0.54-9.5 (n = 47; SD = 1.3). The greatest C/P ratio of all the cases was 9.5 (Case 87). This high ratio is not surprising considering this was a decomposed body so there was ample time for the drugs to move between the tissues and blood. When specimen collection occurs more than 72 hours following death, peripheral blood is preferred for analysis as it is less subject to contamination and redistribution (Wong et al., 2003).

The average C/P ratio from this research project correlates strongly with C/P ratios reported by Ropero-Miller and Winecker (2004). For methadone-related deaths, the C/P ratio was 1.5 for Group 1 (tolerant users) and 1.8 for Group 2 (non-tolerant users). Another source sites a study in which the average C/P ratio was 1.1 (Wong et al., 2003). From published data by Levine et al. (1995), the average heart blood-to-alternate blood ratio for 15 methadone cases was calculated to be 1.3—the same exact ratio seen in this study. In the study by Levine, the alternate blood was subclavian for nine of the cases, pericardial for three of the cases, inferior vena cava for two of the cases, and femoral for just one of the cases. The mean C/P ratio from this research project closely agrees with values from the literature. Methadone is a highly lipophilic drug, so it will readily diffuse from higher concentrations to lower concentrations, redistributing in the body (Drummer, 2004).

Table 18. Ratios of methadone in heart blood to methadone in peripheral blood (femoral) for each case in all groups.

Group	Case	HB/PB(Fem) Ratio for MDN
1A	50	0.54
	65	0.93
	66	0.73
	68	0.83
	74	0.67
	76	0.91
	82	0.56
	89	1.4
1B	19	1.5
	30	1.5
	32	0.80
	34	0.96
	41	0.85
	43	0.85
	49	0.87
	51	1.2
	53	1.6
	73	0.54
	83	1.2
	86	0.82
	91	1.6
2	57	0.80
	72	1.1
	87	9.5
3	33	1.4
	37	1.4
	39	0.86
	45	2.7
	48	1.6
	58	1.1
	64	0.61
	75	0.93
	78	0.94
	84	1.0
	93	3.7
4	42	0.93
	44	1.0
	63	1.0
	67	1.4
	80	1.4
5	79	0.65
	92	0.70
	94	1.6
	95	1.1
	97	1.0
	98	1.2
	99	1.6
Average		1.3
SD		1.3
n		47
Range		0.54-9.5

CONCLUSIONS AND FUTURE WORK

In conclusion, this project has demonstrated the value of analyzing alternative specimens such as brain and liver for methadone-related deaths to determine the distribution of this drug and EDDP. The usefulness of calculating ratios of parent drug to metabolite and concentrations in other matrices has also been assessed. Methadone is a highly lipophilic drug with a high volume of distribution, so it is extensively distributed throughout the body following administration. After dividing the 100 cases into six different groups based on methadone's contribution to death, the mean concentration of methadone and its metabolite were determined for each specimen in each group. Additionally, blood concentrations were related to concentrations in alternative matrices.

Group 1A (drug deaths in which death was attributable to methadone only) had the highest concentration for all specimens except liver. The maximum methadone concentration in liver (19000 ng/g) was actually seen with a case in Group 3 (deaths in which methadone was an incidental finding). Liver concentrations were greater than blood concentrations in all cases. The liver-to-blood ratios ranged from 2.7 to 96 among all groups and were, on average, between five and ten. Brain concentrations were greater than corresponding blood concentrations in all cases. Brain-to-blood ratios ranged from 1.4 to 8.5 among all groups and were, on average, between two and four. The vitreous-to-blood ratios were less than or equal to one in all cases. The maximum mean methadone-to-EDDP ratio in peripheral blood was observed for Group 1A (average = 11; n = 8). No noticeable patterns were observed for these ratios in the other specimens.

It is important to note that Baselt and Cravey (1995) report fatal methadone concentrations of 1.0 mg/L in blood, 1.0 mg/kg in brain, and 3.8 mg/kg in liver. This would equate to a brain-to-blood ratio of 1.0 and a liver-to-blood ratio of 3.8. These results are from a study of only 10 methadone cases and are reported in *Disposition of Toxic Drugs and Chemicals in Man* (a popular reference material among forensic toxicologists). The average brain-to-blood ratios from this research project are all greater than one. Thus, the results from this study of 100 methadone cases could provide an additional reference guide to toxicologists concerning the distribution of methadone in tissues.

Factors such as tolerance and individual differences in metabolism make methadone deaths difficult to interpret due to overlapping therapeutic and toxic concentrations. Drug interactions also complicate interpretation, and additional drug findings are common among methadone deaths. Users will often take other drugs such as benzodiazepines to increase the effects of their highs. These drugs are known to increase respiratory depression when taken with methadone. Individuals who have acquired some tolerance to heroin or morphine can still overdose on methadone since methadone accumulates in the body and does not induce tolerance as readily as other opioids (Garrido and Trocóniz, 1999).

Another problem with interpretation of methadone-related deaths is the phenomenon of postmortem redistribution. The extent of redistribution was evaluated in this study by determining heart blood-to-peripheral blood ratios. The average C/P ratio was 1.3 (n = 47), which is very close to reported values for methadone (Levine et al., 1995; Ropero-Miller and Winecker, 2004). One case in this study had a C/P ratio of 9.5,

which was not unexpected since the decedent was in early stages of decomposition. In other words, there was a long time interval between death and specimen collection in which redistribution occurred.

The findings from this study re-emphasize the importance of obtaining additional information when evaluating the role of methadone in death. There was a considerable overlap of concentrations and ratios between the various groups. Consequently, blood concentrations in isolation are not always useful in interpreting deaths involving methadone. If a blood concentration were ever questionable, then analyzing tissues would help the toxicologist assess the distribution of methadone throughout the body. If the concentrations in these alternative specimens were within the ranges reported in this study, then the toxicologist would be able to better evaluate the blood concentrations.

This research is particularly useful for pathologists and toxicologists in methadone cases where the cause and manner of death are uncertain. This project is not suggesting that additional specimens such as brain and gastric contents be routinely analyzed for methadone cases as this would be ineffective and time-consuming. When applicable though, analysis of alternative specimens may aid in evaluating methadone-related deaths. These cases, however, still remain challenging when little information is known (e.g. dosing history).

Future work could include more in-depth evaluation of drug interactions for each of the specific cases. Additionally, it would be interesting to attempt to retrieve additional case information from methadone clinics in the state. In several cases where high concentrations were unexplainable, it would be valuable to learn of the decedent's dosing history and how long he or she had been taking methadone. This information

would help in assessing the person's probable tolerance. Future work might also involve comparing these postmortem methadone concentrations with concentrations obtained from "Driving Under the Influence" cases in which the methadone users are alive. Comparing concentrations and ratios across these two groups might prove educational.

APPENDIX

Table A1. Information for all 100 cases, including case number, age, sex, race, brief history, cause of death, manner of death, group, and other quantitative results.

Case	Age	Sex	Race	Brief History	COD	MOD	Group	Other Quantitative Results		Sample
1	40	F	C	- Addicted to prescription drugs - History of heroin abuse - Went to MDN clinic earlier that day - Had constant back problems	Overdose of MDN	A	1A	Diphenhydramine	80 ng/mL	HB
2	60	F	C	- Had a lot of back pain recently due to a previous injury - Was on two different prescriptions of morphine	Atherosclerotic Heart Disease	N	3	Diphenhydramine Diazepam Nordiazepam Codeine	80 ng/mL 110 ng/mL 200 ng/mL 400 ng/mL	HB HB HB HB
3	55	F	C	- History of HTN, diabetes, and chronic pain syndrome	Broncho-pneumonia due to cardiac arrhythmia	N	3	Diphenhydramine Fluoxetine Meprobamate	27 ng/mL 160 ng/mL 2.4 mg/L	Blood Blood Blood
4	39	F	C	- Was on a MDN program - Had complained of sleep disturbances	Blunt impact to torso	A	3	None		
5	54	F	C	- History of HTN and chronic back pain - Had morphine pump in abdomen	Obesity	N	3	Nordoxepin	37 ng/mL	PB(Fem)
6	22	M	H	- History of drug abuse including heroin, cocaine, and marijuana	Ligature hanging	S	3	Ethanol	0.075 g/dL	Blood
7	65	M	AA	- History of HTN and chronic back pain - Recently switched from morphine-based medication to MDN	Acute intoxication - multiple drugs	A	1B	Cyclobenzaprine Amitriptyline Nortriptyline Tramadol N-Desmethyltramadol	200 ng/mL 1300 ng/mL 450 ng/mL 1200 ng/mL 200 ng/mL	HB HB HB HB HB
8	42	F	C	- History of drug abuse (specifically cocaine and heroin)	Multiple drug intoxication	A	1B	Ethanol Alprazolam	0.029 g/dL 120 ng/mL	Hosp Serum Hosp Bld

Table A1 (cont'd).

Case	Age	Sex	Race	Brief History	COD	MOD	Group	Other Quantitative Results		Sample
9	43	M	C	- History of sleeping and anxiety disorders - Chronic alcohol abuser	Chronic alcoholism	A	2	Flurazepam Desmethylcitalopram Desalkylflurazepam Oxycodone	23 ng/mL 66 ng/mL 30 ng/mL 200 ng/mL	HB HB HB HB
10	52	F	AA	- History of drug abuse including crack cocaine	Adverse drug reaction to cocaine	A	2	Benzoylecgonine Ecgonine Methyl Ester	200 ng/mL 28 ng/mL	Blood Blood
11	44	F	C	- Was in early stages of decomposition - Recently had heart surgery	Undetermined	U	5	Norfluoxetine Fluoxetine	430 ng/mL 1400 ng/mL	PB(Fem) PB(Fem)
12	41	M	C	- Had back problems due to a fall - Heavy smoker and alcohol abuser - Was part of a pain clinic	ASCAD	N	3	Doxepin Nordoxepin Codeine	200 ng/mL 120 ng/mL 170 ng/mL	HB HB HB
13	38	F	AA	- Had pain in knee - Complained of nausea and vomiting	ASCVD	N	3	None		
14	40	M	C	- History of injuries resulting in back pain	Seizure disorder	N	3	Venlafaxine	64 ng/mL	HB
15	46	M	AA	- Known heroin and crack cocaine abuser	Acute MDN intoxication	A	1A	None		
16	35	M	AA	- History of HTN, asthma, and drug abuse	Asthma	N	3	None		
17	46	M	C	- Was on MDN due to chronic back pain - History of bipolar disorder, Hep C, drug dependence, and seizure disorder - HIV positive	Multiple drug intoxication	A	1B	Diazepam Nordiazepam Temazepam Oxazepam Fentanyl	180 ng/mL 470 ng/mL 190 ng/mL 39 ng/mL 12 ng/mL	HB HB HB HB HB
18	48	F	C	- History of chronic back pain and depression - Was on several prescription drugs	Adverse reaction to multiple drugs	A	1B	Sertraline Desmethylsertraline Promethazine Diphenhydramine Meclizine Hydrocodone	1600 ng/mL 2600 ng/mL 550 ng/mL 240 ng/mL 87 ng/mL 77 ng/mL	Aorta Bld Aorta Bld Aorta Bld Aorta Bld Aorta Bld Aorta Bld

Table A1 (cont'd).

Case	Age	Sex	Race	Brief History	COD	MOD	Group	Other Quantitative Results		Sample
19	32	M	C	- Was seen ingesting handfuls of pills that night	Adverse reaction to drugs	A	1B	Alprazolam	90 ng/mL	HB
20	37	M	AA	- Had recently gone to the emergency room due to pain in his joints associated with sickle cell anemia	Adverse reaction to drugs	A	1B	Cocaine Benzoylecgonine Ecgonine Methyl Ester	49 ng/mL 1800 ng/mL 400 ng/mL	HB HB HB
21	52	M	C	- Was in early stages of decomposition - Chronic alcohol abuser - Had been drinking prior to collapsing	ASCVD	N	3	Ethanol Chlordiazepoxide Nordiazepam Oxazepam	0.21 g/dL 400 ng/mL 610 ng/mL 110 ng/mL	HB HB HB HB
22	29	M	C	- History of heroin abuse	Acute intoxication - multiple drugs	A	1B	Benzoylecgonine Ecgonine Methyl Ester	530 ng/mL 87 ng/mL	HB HB
23	38	M	C	- History of HTN, diabetes, HTCVD, and alcohol abuse	Acute intoxication - multiple drugs	A	1B	Oxycodone	340 ng/mL	HB
24	58	M	C	- History of HTN, heart disease, and alcoholism	Chronic alcoholism	A	4	Ethanol	0.013 g/dL	PB(Fem)
25	55	M	AA	- History of heart problems, HTN, strokes, and bipolar disorder	Acute MDN intoxication	A	1A	Dextromethorphan Doxylamine Hydroxyzine Paroxetine	210 ng/mL 120 ng/mL 760 ng/mL 230 ng/mL	PB(Fem) PB(Fem) PB(Fem) HB
26	45	M	C	- History of depression, anxiety, drug and alcohol abuse, and suicide attempts - Was using cocaine, heroin, and prescription drugs	Chronic pneumonitis	N	3	Nordiazepam Diazepam Ecgonine Methyl Ester Benzoylecgonine	100 ng/mL 59 ng/mL 160 ng/mL 2400 ng/mL	PB(Fem) PB(Fem) PB(Fem) PB(Fem)
27	65	F	C	- History of COPD - Was on several prescription drugs	ASCVD and COPD	N	3	Oxycodone	72 ng/mL	PB(Sub)
28	19	F	C	- Lost control of vehicle and was ejected	Multiple blunt force injuries	A	3	None		
29	50	F	C	- Was diagnosed with bipolar manic depression - Was part of a MDN clinic - History of drug abuse	Acute intoxication - multiple drugs	S	1B	Alprazolam Olanzapine	93 ng/mL 310 ng/mL	PB(Fem) PB(Fem)

Table A1 (cont'd).

Case	Age	Sex	Race	Brief History	COD	MOD	Group	Other Quantitative Results		Sample
30	37	F	C	- History of COPD, HTCVD, and crack cocaine abuse	Adverse reaction to multiple drugs	A	1B	Paroxetine Temazepam Alprazolam	560 ng/mL 22 ng/mL 42 ng/mL	PB(Fem) HB HB
31	43	M	C	- Had lower back brace due to motor vehicle accident 10 years ago	Lobar pneumonia	N	3	None		
32	49	F	AA	- Was a recovering heroin addict	Adverse reaction to multiple drugs	A	1B	Cocaine Ecgonine Methyl Ester	170 ng/mL 260 ng/mL	PB(Fem) PB(Fem)
33	44	F	C	- History of depression, anxiety, sleep apnea, and severe asthma	COPD	N	3	None		
34	38	M	C	- Was on MDN up to 3 times daily for chronic back pain	Adverse effects of multiple drugs	A	1B	None		
35	22	M	AA	- History of drug abuse including cocaine	Acute intoxication - multiple drugs	A	1B	Benzoylecgonine	220 ng/mL	Hosp Bld
36	58	M	AA	- History of COPD - Went to MDN clinic every morning	Acute MDN intoxication	A	1A	None		
37	50	F	C	- History of back pain and crack cocaine abuser - Heavy smoker	Broncho-pneumonia	N	3	Nordiazepam Diazepam Ecgonine Methyl Ester Benzoylecgonine	330 ng/mL 290 ng/mL 44 ng/mL 810 ng/mL	PB(Fem) PB(Fem) PB(Fem) PB(Fem)
38	21	M	C	- History of drug abuse for 6 years	Adverse effects of alpha methyltryptamine	A	2	Delta-9 carboxy THC	6.5 ng/mL	HB
39	55	M	C	- History of Hep C, COPD, and abuse of over-the-counter medications	COPD with emphysema and fibrosis	N	3	None		
40	48	M	C	- Was on several prescription drugs - Previous suicide attempts	ASCVD complicated by adverse reaction to drugs	A	4	Ethanol Nordiazepam Oxazepam Temazepam Diazepam Benzoylecgonine	0.095 g/dL 1100 ng/mL 120 ng/mL 170 ng/mL 1100 ng/mL 170 ng/mL	PB(Fem) PB(Fem) PB(Fem) PB(Fem) PB(Fem) PB(Fem)

Table A1 (cont'd).

Case	Age	Sex	Race	Brief History	COD	MOD	Group	Other Quantitative Results		Sample
41	51	F	C	- History of prescription drug abuse, insomnia, Hep C, and depression - Traded her medications with others - Was on MDN	Adverse reaction to multiple drugs	A	1B	Amitriptyline Paroxetine Diphenhydramine Nortriptyline Cocaine Benzoylecgonine	330 ng/mL 250 ng/mL 430 ng/mL 440 ng/mL 27 ng/mL 85 ng/mL	PB(Fem) PB(Fem) PB(Fem) PB(Fem) PB(Fem) PB(Fem)
42	50	M	C	- History of COPD, IV drug abuse, Hep C, and opiate dependency - Heavy smoker	COPD aggravated by multiple drugs	A	4	Nordiazepam Ecgonine Methyl Ester Cocaine Benzoylecgonine Morphine	44 ng/mL 470 ng/mL 41 ng/mL 4100 ng/mL 43 ng/mL	PB(Fem) PB(Fem) PB(Fem) PB(Fem) PB(Fem)
43	40	M	C	- History of spina bifida, HTCVD, and heroin and alcohol abuse - Was part of a MDN clinic	Adverse effects of multiple drugs	A	1B	Ethanol Oxazepam Nordiazepam	0.22 g/dL 30 ng/mL 56 ng/mL	PB(Fem) PB(Fem) PB(Fem)
44	48	F	C	- Chronic drug and alcohol addict - Recently admitted to a MDN clinic - History of Hep C and COPD	ASCAD complicated by drugs	N	4	Amitriptyline Nortriptyline Chlorpromazine Doxepin Phenobarbital	190 ng/mL 130 ng/mL 360 ng/mL 100 ng/mL 10 mg/L	HB HB HB HB HB
45	43	M	C	- History of heavy tobacco abuse, COPD, HTCVD, obesity, and sleep apnea	ASCVD and HCVD	N	3	Nordiazepam Diazepam Ecgonine Methyl Ester Cocaine Benzoylecgonine Oxycodone	90 ng/mL 100 ng/mL 170 ng/mL 92 ng/mL 1300 ng/mL 65 ng/mL	PB(Fem) PB(Fem) PB(Fem) PB(Fem) PB(Fem) PB(Fem)

Table A1 (cont'd).

Case	Age	Sex	Race	Brief History	COD	MOD	Group	Other Quantitative Results		Sample
46	46	M	C	- Was in advanced stages of decomposition - History of prescription drug abuse, alcohol abuse, depression, and anxiety - Had Lupus	Adverse reaction to multiple drugs	A	1B	Ethanol Diphenhydramine Phenobarbital Alpha-hydroxyalprazolam Cocaine Benzoylecgonine Ecgonine Methyl Ester Cocaethylene Fentanyl	0.089 g/100g 870 ng/g 4 mg/kg 280 ng/g 160 ng/g 930 ng/g 200 ng/g 210 ng/g 11 ng/g	Liver Liver Liver Liver Liver Liver Liver Liver Liver
47	55	F	AA	- Was embalmed - History of alcohol abuse	Undetermined	U	5	Ethanol	0.037 g/dL	Hosp Serum
48	34	F	AA	- History of asthma - Was on MDN for heroin and cocaine addiction	Ruptured ectopic pregnancy	N	3	Ecgonine Methyl Ester Benzoylecgonine	64 ng/mL 370 ng/mL	PB(Fem) PB(Fem)
49	48	F	AA	- History of chronic alcohol and cocaine abuse and congestive heart failure - HIV positive	Adverse effects of multiple drugs	A	1B	Ecgonine Methyl Ester Cocaine Benzoylecgonine	530 ng/mL 150 ng/mL 3500 ng/mL	PB(Sub) PB(Sub) PB(Sub)
50	16	F	AA	- History of depression and suicide attempts	Acute MDN intoxication	A	1A	None		
51	24	M	C	- History of depression and drug abuse	Adverse reaction to multiple drugs	A	1B	Alprazolam Ecgonine Methyl Ester Cocaine Benzoylecgonine	31 ng/mL 290 ng/mL 41 ng/mL 4000 ng/mL	PB(Fem) PB(Fem) PB(Fem) PB(Fem)
52	45	M	C	- History of back pain for 10 years -History of pain medication and alcohol abuse	HTCVD complicated by drugs	A	4	Fentanyl	4.4 ng/mL	PB(Fem)
53	39	M	C	- History of drug abuse including cocaine for 20 years	Adverse reaction to multiple drugs	A	1B	Ecgonine Methyl Ester Benzoylecgonine	96 ng/mL 1400 ng/mL	PB(Fem) PB(Fem)
54	35	M	AA	- Was drinking and using heroin that day	Adverse reaction to multiple drugs	A	1B	Ethanol Benzoylecgonine	0.10 g/dL 210 ng/mL	PB(Sub) PB(Sub)

Table A1 (cont'd).

Case	Age	Sex	Race	Brief History	COD	MOD	Group	Other Quantitative Results		Sample
55	46	M	C	- Was diagnosed as bipolar - History of prescription drug abuse	Adverse reaction to multiple drugs	A	1B	Ethanol Nordiazepam Diazepam Alprazolam	0.01 g/dL 87 ng/mL 180 ng/mL 120 ng/mL	HB PB(Sub) PB(Sub) PB(Sub)
56	34	M	C	- History of heroin abuse	Adverse reaction to multiple drugs	A	1B	Ecgonine Methyl Ester Benzoylecgonine	88 ng/mL 1200 ng/mL	PB(Sub) PB(Sub)
57	46	F	C	- Was part of a MDN clinic	Adverse reaction to opiates and ethanol	A	2	Ethanol Amitriptyline Nortriptyline	0.13 g/dL 650 ng/mL 310 ng/mL	PB(Fem) PB(Fem) PB(Fem)
58	17	M	C	- Previous suicide attempts - Was part of a MDN clinic for heroin abuse	Gunshot wound to head	S	3	None		
59	44	M	C	- Chronic back pain for 4 years - Was taking several prescription drugs	Adverse effects of MDN	A	1A	None		
60	27	M	C	- Was on MDN for heroin abuse - Smoked cigarettes dipped in formaldehyde	Asphyxia due to hanging	S	3	Morphine Phencyclidine	370 ng/mL 210 ng/mL	PB(Sub) PB(Sub)
61	47	M	C	- History of HTCVD and alcohol and tobacco abuse	ASCVD and HCVD	N	3	Ethanol	0.12 g/dL	HB
62	56	M	C	- History of alcohol abuse, diabetes, HTN, and heavy smoking - Stole MDN pills	ASCVD complicated by drugs	A	4	Amitriptyline Nortriptyline Venlafaxine Desmethylvenlafaxine Butalbital Alprazolam	9100 ng/mL 580 ng/mL 800 ng/mL 300 ng/mL 0.98 mg/L 110 ng/mL	PB(Sub) PB(Sub) PB(Sub) PB(Sub) PB(Sub) PB(Sub)
63	24	M	C	- History of IV drug abuse including heroin	ASCAD - MDN contributory	A	4	None		
64	46	M	C	- History of depression, suicide attempts, alcohol and prescription drug abuse, and chronic pain	ASCVD, HCVD, and cirrhosis	N	3	Nordiazepam Diazepam Temazepam	620 ng/mL 280 ng/mL 22 ng/mL	PB(Fem) PB(Fem) PB(Fem)

Table A1 (cont'd).

Case	Age	Sex	Race	Brief History	COD	MOD	Group	Other Quantitative Results		Sample
65	40	M	AA	- Was a recovering heroin addict and recently started MDN treatments - Went to MDN clinic the previous day	Acute MDN intoxication	A	1A	None		
66	27	F	C	- History of IV drug abuse including heroin - Was on MDN for back pain	Adverse reaction to MDN	A	1A	Temazepam	48 ng/mL	PB(Fem)
67	51	M	C	- History of chronic back pain and HTN	ASCVD and HCVD complicated by MDN	A	4	None		
68	32	F	C	- History of bipolar disorder, chronic pain, depression, and suicide attempts - Heavy smoker	Acute MDN intoxication	S	1A	None		
69	41	M	C	- Recently had migraines and was under a lot of stress	Acute hemorrhagic pancreatitis	N	3	Nordiazepam Diazepam	60 ng/mL 71 ng/mL	Blood Blood
70	40	M	C	- History of asthma - Complained of nausea and difficulty breathing	Interstitial pneumonia	N	3	Alprazolam Morphine	51 ng/mL 47 ng/mL	HB HB
71	49	M	C	- History of HTN and drug abuse	Hypertension	N	3	None		
72	38	M	C	- Was part of a MDN clinic - History of crack cocaine abuse	Adverse reaction to crack cocaine	A	2	Benzoylecgonine Cocaine Ecgonine Methyl Ester	5700 ng/mL 400 ng/mL 1000 ng/mL	PB(Fem) PB(Fem) PB(Fem)
73	50	M	C	- History of alcohol and prescription pain medication abuse	Acute drug intoxication	A	1B	Ethanol Oxycodone	0.17 g/dL 69 ng/mL	PB(Fem) PB(Fem)
74	23	M	C	-History of prescription drug abuse - Purchased MDN tablets from an an unknown source	Acute MDN intoxication	A	1A	None		
75	35	F	C	- History of bipolar disorder and heroin abuse - Went to MDN clinic daily	ASCVD	N	3	None		

Table A1 (cont'd).

Case	Age	Sex	Race	Brief History	COD	MOD	Group	Other Quantitative Results		Sample
76	53	M	C	- History of injuries and accidental medication overdoses - Was on MDN for lower back pain	Adverse reaction to MDN	A	1A	None		
77	49	F	C	- Was under a doctor's care for a bladder problem and ear infection	Adverse reaction to drugs	A	1B	Oxycodone	29 ng/mL	PB(Sub)
78	43	M	C	- History of bipolar disorder, depression, and suicide attempts - History of drug abuse including crack cocaine and prescription drugs	ASCAD and HCVD	N	3	None		
79	45	F	C	- History of depression and intentional overdoses	Cardiac dysrhythmia	U	5	Sertraline Bupropion Citalopram Desmethylcitalopram Phenobarbital	240 ng/mL 120 ng/mL 500 ng/mL 90 ng/mL 0.92 mg/L	PB(Fem) PB(Fem) PB(Fem) PB(Fem) HB
80	23	M	C	- Had viral meningitis - Complained of vomiting and headaches	Broncho-pneumonia aggravated by drugs	A	4	None		
81	53	F	AA	- History of HTN, Hep C, and cirrhosis - Was on MDN for heroin addiction - Complained of vomiting and dizziness	Hep C cirrhosis	N	3	None		
82	38	F	C	- Was on MDN for chronic back pain	Acute MDN intoxication	A	1A	None		
83	28	M	C	- History of cocaine and heroin abuse	Adverse reaction to multiple drugs	A	1B	Ecgonine Methyl Ester Benzoylecgonine	120 ng/mL 980 ng/mL	PB(Fem) PB(Fem)
84	44	M	C	- History of shoulder pain, Hep C, and asthma - Heavy smoker	Pneumonia and COPD	N	3	None		
85	30	M	C	- Careless smoking considered the probable cause	Smoke inhalation and thermal burns	A	3	Carboxyhemoglobin	51 %COHb	HB

Table A1 (cont'd).

Case	Age	Sex	Race	Brief History	COD	MOD	Group	Other Quantitative Results		Sample
86	35	M	AA	- History of depression and HTN - Had been vomiting blood	Adverse reaction to multiple drugs	A	1B	Venlafaxine Desmethylvenlafaxine Ecgonine Methyl Ester Benzoylecgonine	300 ng/mL 140 ng/mL 84 ng/mL 510 ng/mL	PB(Fem) PB(Fem) PB(Fem) PB(Fem)
87	50	F	C	- Was in early stages of decomposition - Had several back surgeries - History of depression	Multiple drug intoxication	U	2	Ethanol Oxycodone	0.016 g/dL 45 ng/mL	HB PB(Fem)
88	37	F	C	- Was exhumed due to possible poisoning	Pending	P	5	Ethanol Methanol	0.073 g/dL 0.062 g/dL	Brain Brain
89	23	M	C	- History of alcohol, cocaine, and heroin abuse	Adverse reaction to MDN	A	1A	None		
90	58	M	C	- History of depression and Hep C	Gunshot wound to the head	S	3	Ethanol 6-Acetylmorphine Codeine Morphine	0.23 g/dL 15 ng/mL 50 ng/mL 470 ng/mL	PB(Sub) PB(Sub) PB(Sub) PB(Sub)
91	44	F	C	- Went to MDN clinic daily for heroin abuse - History of depression	Combined drug toxicity	S	1B	Ethanol Ecgonine Methyl Ester Cocaine Benzoylecgonine	0.032 g/dL 490 ng/mL 62 ng/mL 2300 ng/mL	PB(Fem) PB(Fem) PB(Fem) PB(Fem)
92	24	F	C	- History of bipolar disorder and suicide attempts - Many MDN pills of a recent prescription were missing	Pending	P	5	None		
93	22	F	C	- Driver was under the influence of drugs and alcohol	Blunt force trauma	A	3	Ethanol Meprobamate	0.11 g/dL 6.1 ng/mL	PB(Fem) PB(Fem)
94	24	M	C	- History of drug and alcohol abuse - Recently purchased MDN from a friend	Pending	P	5	Ecgonine Methyl Ester Benzoylecgonine	89 ng/mL 590 ng/mL	PB(Fem) PB(Fem)
95	53	F	C	- Not available	Pending	P	5	Norpropoxyphene	340 ng/mL	PB(Fem)

Table A1 (cont'd).

Case	Age	Sex	Race	Brief History	COD	MOD	Group	Other Quantitative Results	Sample
96	54	M	C	- Recently diagnosed with acute bronchitis	Pending	P	5	Testing not completed	
97	32	M	C	- Not available	Pending	P	5	Testing not completed	
98	43	M	C	- History of IV drug abuse, accidental overdoses including crack cocaine and prescription drugs	Pending	P	5	Testing not completed	
99	33	F	C	- History of prescription drug abuse	Pending	P	5	Testing not completed	
100	50	F	C	- History of chronic pain, depression, arthritis, back spasms, COPD, and HTCVD - Heavy smoker	Pending	P	5	Testing not completed	

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