2.2 Case Selection

Selection of cases was dependent on the circumstances of death. The forensic pathologist concentrated on those decedents whose past medical history or findings at the scene suggested a drug-related death or who were likely to have prescription medications or illicit drugs in their blood at the time of death. Decedents who were victims of homicide or suspected homicide were excluded from the study. If postmortem acquisition of blood was insufficient for both routine toxicology testing and testing as part of the research project because of trauma or other causes of hypovolemia, these decedents were excluded from the study. Decedents undergoing decomposition were also excluded.

2.3 Informed Consent

Decedents are not considered "human subjects" for research purposes. Postmortem blood is normally obtained from various sites as part of a routine forensic autopsy, so no fluids or tissues not ordinarily retained occurred. In addition, toxicology tests are normally performed on postmortem blood or tissues as part of the standard forensic autopsy by the Iowa Office of the State Medical Examiner. Based on these conditions, this study was considered exempt from review by an IRB committee. Iowa code permits the retention of significant portions of tissues for diagnostic, research and teaching purposes without notification of next-of-kin (Iowa Code § 691.6(8)(2005)). Despite the generous allowances of the code to conduct research without the need for informed consent, the researchers felt it was just and reasonable to pursue informed consent from the legal next-of-kin or representative prior to inclusion of a particular decedent's specimens into this study. Informed consent was generally obtained by the research assistant whose salary was funded by this grant via telephone contact with the identified legal next-of-kin or representative.

2.4 Specimen Collection

Routine autopsy specimens included heart blood collected by visually directed stick of the inferior vena cava, femoral blood collected by a "blind" inguinal stick or by direct visualization of the iliac vein with or without massaging of the leg depending on ease of acquiring blood, urine and vitreous fluid. All blood specimens were collected prior to evisceration. Of these routine specimens, only the femoral blood specimen acquired by "blind" stick of the inguinal region (Specimen 1) was evaluated in this study. This specimen was placed in a gray-top Vacutainer® tube containing sodium fluoride and sent to AIT for testing as part of the routine casework. These samples were refrigerated but not frozen until they were shipped in standard cardboard box containers with thin Styrofoam insulation provided by AIT. Samples were held in refrigeration prior to shipping no more than 2 days after acquisition. The specimens were shipped in ambient conditions via a commercial courier (FedEx) by plane from Des Moines, IA, which is 20 minutes from the IOSME in Ankeny, IA, through the main FedEx hub in Memphis, TN to Indianapolis, IN prior to ground delivery to AIT. According to a service representative for FedEx, ambient temperatures on the planes vary according to type of plane, package location on the plane, and the cruising altitude. In general, most temperatures in flight are between 65 and 90°F; however, lower cargo and bulk on certain planes can reduce the temperature to 0 degrees Fahrenheit. On arrival at AIT, the specimens were accessioned, refrigerated during testing and placed in long-term -20°Cstorage.

On the selected cases, two additional peripheral blood specimens were obtained. Both specimens were collected and immediately placed in a gray-top Vacutainer® tube, containing sodium fluoride. Iliac blood (Specimen 2) was obtained by opening the abdominal cavity, isolating the iliac vein on the opposite side from which the femoral vein specimen had been

taken by clamping its proximal and distal segments, and aspirating any blood by direct visualization prior to any external aspiration of blood or manipulation of the legs. This specimen was immediately frozen by placement in a -60°C freezer (maintained consistently at -57°C) prior to transport to the reference laboratory. This specimen was shipped on dry ice to maintain its frozen state. After the blood from the iliac vein had been obtained, an external "blind stick" of the inguinal region from the same side as the femoral venous sample (Specimen 1) with or without massaging of the right leg and aspiration of presumed femoral venous blood was performed (Specimen 3). This specimen was shipped on dry ice to maintain its frozen state to the reference laboratory. Specimens 2 and 3 were maintained at -57°C until shipment to AIT Laboratories until results from the routine autopsy specimens had been received and informed consent had been obtained. If the case was selected for inclusion into the study, Specimens 2 and 3 were submitted to AIT Laboratories for testing. On average, 5.7 mL (range 2.0 – 10.0 mL) of blood was collected for Specimens 2 and 3.

Cases were given a second identification number in addition to the standard case identification label. The cases were labeled sequentially as they were entered into the study. The first case was labeled, "R-001," the second case was labeled, "R-002," the third case was labeled, "R-003," and so forth. In addition, the blood obtained from the clamped iliac vein was given the suffix of "-A." The research blood from the right leg that was frozen was given the suffix of "-B." For example, blood obtained from the clamped iliac vein from the first case was labeled, "R-001-A."

2.5 Testing

Specimens were analyzed at AIT according to standard operating procedures. Classical cannabinoids, opiates, and oxycodone/metabolite were screened by an enzyme linked

Waters Quattro Premier XE tandem quadrupole mass spectrometer (UPLC/MS/MS). Acetaminophen, carbamazepine, carbamazepine-10,11-epoxide, citalopram, lamotrigine, promethazine, and trazodone were confirmed and quantified on a Waters Alliance 2695 high performance liquid chromatograph with either a Waters 2487 ultraviolet detector or a Waters 2475 fluorescence detector (HPLC). Butalbital was confirmed and quantified on an Agilent 6890 gas chromatograph coupled to an Agilent 5937 mass spectrometer (GC/MS). Ethanol was confirmed and quantified on a Hewlett Packard 5890 gas chromatography with flame ionization detector (GC-FID). Carbon monoxide was confirmed and quantified on an IL-682 CO-Oximeter (CO-OX).

All analytical methods utilized were validated according to laboratory standard operating procedures. Parameters assessed during method validation for qualitative screening assays included limit of detection (LOD), imprecision and accuracy at the analytical cutoff, matrix selectivity, exogenous drug interferences, and carryover. Parameters assessed during method validation for quantitative confirmatory assays included linearity, limit of detection (LOD), lower limit of quantitation (LLOQ), and upper limit of quantitation (ULOQ), imprecision and accuracy, matrix selectivity, exogenous drug interferences, ion suppression (when necessary), and carryover. Analytical cutoffs were determined during validation testing and were relevant to postmortem toxicology.

2.6 Statistical Methods

Data were analyzed by repeated measures analysis of variance (ANOVA, $p < 0.05$) using EZAnalyze© 3.0, statistical analysis software for Microsoft® Excel. Collection/shipping procedure was the independent variable and drug concentration was the dependent variable.

3. RESULTS

3.1 Demographics

Femoral, iliac and inguinal specimens (438 total) were collected from 146 decedents during the period from January 2011 to February 2013. The cause of death was attributed to a single drug in 17 cases (12%), a mixture of multiple drugs in 51 cases (35%) inhalation of carbon monoxide in 2 cases (1%), inhalation of paint thinner in 1 case (1%) and inhalation of chloroform in 1 case (1%). Drugs were considered to be a significant other condition that contributed to death in 13 cases (9%). The manners of death were certified as follows: accidental (42%), natural (24%), suicide (23%) and undetermined (11%). The age range was 13 to 80 years old (mean age 42 years old). There were 91 males (62%) and 54 females (37%). Caucasians represented 96% of the subjects and African Americans represented 6%. The weight range was from 104 to 300 pounds (mean 195 pounds). The average postmortem interval, defined as time last seen alive to time of specimen collection, was 39 hours. Obtaining the initial femoral blood specimen by vigorous massaging was required in only 11 cases.

3.2 Drugs Detected

Of the 146 cases in which specimens had been collected, there were 112 cases in which 62 different illicit, prescription and over-the-counter drugs and/or their metabolites were detected (Table 1). A single specimen triplet is defined as femoral, iliac and inguinal specimens, collected from a single decedent, that were analyzed for a single drug. Multiple drugs and or metabolites were detected in most subjects. Sixteen drugs/metabolites were detected in a single specimen triplet each (Table 2). For the remaining drugs/metabolites, there were 2-37 specimen triplets analyzed.

3.3 Statistical Findings

Only delta-9-carboxy-THC (cannabis metabolite THCCOOH, *n = 12, p = 0.021*), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (methadone metabolite, EDDP, *n = 9, p = 0.001*), and amphetamine (*n = 5, 0.021*) concentrations were significantly different between the three collection/shipping procedures ($p < 0.05$). Post Hoc analyses revealed that the inguinal specimen shipped on dry ice (Specimen 3) had significantly lower analyte concentrations than Specimen 1 or Specimen 2 for THCCOOH, EDDP and amphetamine. For amphetamine, inguinal blood was significantly lower than iliac blood. Trends toward significance ($p < 0.10$) were determined for nortiptyline (*n = 10, p = 0.065*), N-desmethyltramadol (*n = 8, p = 0.074*), buprenorphine (*n = 3, p = 0.067*) and norbuprenorphine (*n = 2, p = 0.067*).

Table 1: Summary of drugs and/or metabolite concentrations (ng/mL) in 112 decedents (457 specimen triplets). Femoral blood was collected by blind stick and shipped to the laboratory at ambient temperature for comprehensive toxicology panel. Iliac blood was collected by direct visualization and clamping the vein. Inguinal blood was collected by blind stick of the femoral vein. Iliac and Inguinal blood were shipped to the laboratory on dry ice for comprehensive toxicology panel.

DRUG/METABOLITE	*N*	BLOOD	MEAN ± SD	MEDIAN	RANGE	*P*
Alprazolam	37	Femoral	54.9 ± 68.7	37.7	2.5 - 364.0	.536
		Iliac	52.4 ± 54.1	36.7	2.6 - 229.0	
		Inguinal	50.7 ± 51.5	35.9	3.5 - 228.0	
7-aminoclonazepam	21	Femoral	41.6 ± 31.6	33.5	42.9 - 114.0	.536
		Iliac	47.4 ± 34.6	48.2	21.0 - 162.0	
		Inguinal	50.5 ± 36.3	38.0	18.5 -169.0	
Morphine	19	Femoral	282.9 ± 232.5	226.0	31.5 - 863.0	.424
		Iliac	345.1 ± 339.8	239.0	24.0 - 1413.0	
		Inguinal	279.2 ± 243.4	203.0	27.6 - 953.0	
Oxycodone	16	Femoral	227.3 ± 300.7	100.0	10.1 - 1137.0	.565
		Iliac	220.3 ± 261.6	100.4	11.3 - 934.0	
		Inguinal	195.7 ± 200.7	103.3	0.0 - 526.0	
Ethanol (%w/v)	15	Femoral	0.15 ± 0.13	0.09	0.03 - 0.43	.890
		Iliac	0.15 ± 0.13	0.10	0.03 - 0.47	
		Inguinal	0.15 ± 0.13	0.10	0.03 - 0.45	
Diphenhydramine	14	Femoral	3165.5 ± 6072.5	369.0	56.5 - 19733.0	.212
		Iliac	3304.7 ± 6450.0	269.0	53.3 - 18468.0	
		Inguinal	2441.1 ± 4549.1	326.0	0.0 - 12761.0	

DRUG/METABOLITE	*N*	BLOOD	MEAN ± SD	MEDIAN	RANGE	*P*
Citalopram	14	Femoral	356.2 ± 534.1	148.5	10.5 - 1742.0	.306
		Iliac	444.3 ± 591.5	202.5	34.0 - 1960.0	
		Inguinal	269.5 ± 324.0	175.0	24.1 - 1229.0	
THC	14	Femoral	3.4 ± 3.4	1.8	1.0 - 12.6	.241
		Iliac	1.5 ± 1.4	1.4	0.0 - 4.2	
		Inguinal	1.8 ± 4.2	0.0	0.0 - 15.3	
Methadone	13	Femoral	363.2 ± 216.7	341.0	33.0 - 849.0	.662
		Iliac	344.1 ± 198.7	308.0	30.0 - 653.0	
		Inguinal	338.9 ± 226.1	271.0	25.0 - 885.0	
THC-COOH	12	Femoral	23.0 ± 19.6	15.2	5.0 - 64.9	***.021***
		Iliac	20.0 ± 14.8	16.9	4.6 - 50.4	
		Inguinal	15.0 ± 11.2	11.7	4.1 - 45.3	
Hydrocodone	12	Femoral	372.7 ± 992.8	35.6	13.1 - 3512.0	.349
		Iliac	236.1 ± 454.8	43.5	17.9 -1639.0	
		Inguinal	150.1 ± 293.7	35.5	15.8 - 1062.0	
Nordiazepam	12	Femoral	341.9 ± 236.4	286.0	62.0 -688.0	.981
		Iliac	360.9 ± 211.0	336.0	68.4 -732.0	
		Inguinal	357.3 ± 202.0	319.0	93.6 - 613.0	
Fluoxetine	11	Femoral	385.1 ± 420.6	189.0	24.8 - 1431.0	.121
		Iliac	168.1 ± 197.7	98.6	0.0 - 562.0	
		Inguinal	88.9 ± 116.2	0.0	0.0 - 270.0	
Diazepam	10	Femoral	287.1 ± 182.3	303.0	59.8 - 640.0	.386
		Iliac	267.9 ± 157.5	295.0	0.0 - 532.0	
		Inguinal	288.5 ± 168.3	301.5	0.0 - 627.0	
Nortriptyline	10	Femoral	245.8 ± 149.7	253.0	41.4 - 478.0	.065
		Iliac	185.0 ± 187.3	81.3	0.0 - 493.0	
		Inguinal	149.5 ± 131.1	116.2	0.0 - 339.0	
Amitriptyline	9	Femoral	1206.6 ± 1826.6	357.0	23.3 - 5324.0	.597
		Iliac	850.4 ± 1494.3	316.0	20.3 - 4749.0	
		Inguinal	744.6 ± 1257.4	316.0	0.0 - 4000.0	
Fentanyl	9	Femoral	8.8 ± 5.9	7.1	1.9 - 21.1	.714
		Iliac	7.3 ± 7.1	5.7	0.0 - 23.9	
		Inguinal	8.4 ± 4.7	10.1	1.7 - 13.9	
EDDP	9	Femoral	51.2 ± 27.0	51.0	27.8 - 116.0	***.001***
		Iliac	50.5 ± 26.6	46.8	25.4 - 113.0	
		Inguinal	34.7 ± 24.5	37.1	0.0 - 74.9	
Acetaminophen	8	Femoral	90.3 ± 130.2	36.3	4.3 - 395.0	.271
(mg/L)		Iliac	84.3 ± 105.2	38.7	4.9 - 308.0	
		Inguinal	63.6 ± 70.4	33.1	3.5 - 213.0	
Gabapentin	8	Femoral	22.9 ± 20.4	19.3	1.6 -56.30	.528
(mg/L)		Iliac	26.6 ± 25.1	23.7	2.1 - 75.8	
		Inguinal	21.6 ± 15.0	24.2	2.1 - 39.3	

DRUG/METABOLITE	*N*	BLOOD	MEAN ± SD	MEDIAN	RANGE	*P*
Nortramadol	8	Femoral	383.4 ± 318.5	270.5	139.0 -1081.0	.074
		Iliac	493.5 ± 426.0	391.5	151.0 - 1434.0	
		Inguinal	452.1 ± 303.8	390.0	146.0 - 1122.0	
Paroxetine	7	Femoral	615.1 ± 938.3	400.0	27.4 - 2695.0	.395
		Iliac	679.0 ± 1149.4	401.0	32.6 - 3254.0	
		Inguinal	346.4 ± 329.0	242.0	31.6 - 907.0	
Tramadol	7	Femoral	3281.6 ± 4370.2	1092.0	280.0 - 11869.0	.528
		Iliac	3151.0 ± 4125.1	1296.0	0.0 - 11429.0	
		Inguinal	2880.1 ± 3297.6	2091.0	0.0 - 9013.0	
Hydromorphone	7	Femoral	11.7 ± 10.5	5.1	2.6 -25.3	.502
		Iliac	11.2± 11.0	4.4	0.0 - 25.6	
		Inguinal	13.6 ± 12.1	18.1	0.0 - 30.2	
Methamphetamine	7	Femoral	442.7 ± 514.7	170.0	71.0 -1253.0	.117
		Iliac	572.0 ± 721.9	177.0	75.3 -1745.0	
		Inguinal	412.7 ± 477.5	160.0	83.8 -1242.0	
Norfluoxetine	6	Femoral	254.2 ± 119.4	239.5	121.0 -441.0	.135
		Iliac	157.0 ± 102.7	126.3	39.5 - 284.0	
		Inguinal	123.5 ± 141.2	81.6	0.0 - 343.0	
Quetiapine	6	Femoral	260.1 ± 153.1	258.0	90.6 - 480.0	.308
		Iliac	705.4 ± 954.6	429.0	77.5 - 2613.0	
		Inguinal	442.3 ± 413.3	354.5	62.1 - 1180.0	
Amlodipine	6	Femoral	187.8 ± 404.4	24.8	9.9 - 1013.0	.372
		Iliac	319.2 ± 699.7	37.0	0.0 - 1746.0	
		Inguinal	177.0 ± 359.7	35.9	7.0 - 910.0	
Cyclobenzaprine	6	Femoral	146.8 ± 133.6	86.6	33.6 - 375.0	.545
		Iliac	148.2 ± 177.7	70.9	30.7 - 490.0	
		Inguinal	168.9 ± 179.7	103.9	47.9 - 523.0	
Lamotrigine	6	Femoral	3.0 ± 1.2	3.1	1.4 - 4.5	.133
(mg/L)		Iliac	2.6 ± 0.7	2.6	1.5 - 3.4	
		Inguinal	2.2 ± 0.9	2.0	1.4 - 3.6	
Lorazepam	6	Femoral	62.7 ± 24.9	55.6	33.4 - 93.1	.804
		Iliac	60.2 ± 29.6	62.4	19.6 - 103.0	
		Inguinal	70.1 ± 45.3	58.0	22.3 - 140.0	
Metoprolol	6	Femoral	153.5 ± 127.9	105.9	42.2 - 353.0	.457
		Iliac	171.2 ± 133.4	133.8	57.4 - 362.0	
		Inguinal	150.9 ± 112.5	107.9	56.1 - 313.0	
Mirtazapine	6	Femoral	118.2 ± 85.5	120.5	26.1 - 226.0	.311
		Iliac	109.4 ± 100.2	83.7	28.7 - 284.0	
		Inguinal	135.3 ± 115.8	124.0	29.1 - 276.0	
Pregabalin	6	Femoral	10.0 ± 11.8	6.7	2.1 -33.7	.994
(mg/L)		Iliac	10.1 ± 14.8	5.8	0.6 - 39.9	
		Inguinal	10.0 ± 14.3	5.8	0.6 - 38.8	

DRUG/METABOLITE	*N*	BLOOD	MEAN ± SD	MEDIAN	RANGE	*P*
Temazepam	6	Femoral	233.8 ± 145.0	205.0	74.8 - 411.0	.878
		Iliac	239.0 ± 168.8	193.5	75.2 -486.0	
		Inguinal	253.3 ± 160.7	278.5	62.0 - 408.0	
Zolpidem	6	Femoral	451.9 ± 700.3	116.1	24.8 - 1819.0	.275
		Iliac	512.3 ± 904.5	121.0	21.6 - 2336.0	
		Inguinal	367.4 ± 653.5	69.6	32.4 - 1684.0	
Amphetamine	5	Femoral	192.5 ± 103.8	160.0	65.4 - 303.0	***.021***
		Iliac	236.7 ± 137.6	233.0	66.4 - 438.0	
		Inguinal	155.1 ± 119.9	147.0	0.0 - 291.0	
Carbamazepine	5	Femoral	12.8 ± 10.2	10.3	2.9 - 30.1	.454
(mg/L)		Iliac	12.8 ± 11.1	9.9	2.7 - 31.6	
		Inguinal	14.8 ± 15.2	9.3	2.5 - 41.1	
Duloxetine	5	Femoral	149.9 ± 163.6	66.8	54.6 - 437.0	.348
		Iliac	173.6 ± 244.0	65.7	57.6 - 610.0	
		Inguinal	110.8 ± 139.4	65.9	0.0 - 354.0	
Norsertraline	5	Femoral	932.6 ± 1067.4	373.0	162.0 - 2750.0	.109
		Iliac	112.6 ± 163.9	0.0	0.0 - 360.0	
		Inguinal	94.0 ± 134.7	0.0	0.0 - 291.0	
Sertraline	5	Femoral	1036.8 ± 1482.5	201.0	133.0 - 3577.0	.112
		Iliac	367.4 ± 821.5	0.0	0.0 - 1837.0	
		Inguinal	279.2 ± 454.3	139.0	0.0 - 1079.0	
Bupropion	4	Femoral	367.0 ± 421.5	163.0	143.0 - 999.0	.462
		Iliac	388.0 ± 490.0	145.5	138.0 - 1123.0	
		Inguinal	420.3 ± 521.8	183.0	116.0 - 1199.0	
Dextromethorphan	4	Femoral	78.1 ± 87.1	44.7	16.2 - 207.0	.150
		Iliac	99.4 ± 110.8	54.0	25.8 - 264.0	
		Inguinal	75.5 ± 86.9	51.1	0.0 - 200.0	
Promethazine	3	Femoral	116.7 ± 110.3	82.9	27.3 - 240.0	.142
		Iliac	73.3 ± 127.0	0.0	0.0 - 220.0	
		Inguinal	79.3 ± 137.4	0.0	0.0 - 238.0	
Benzoylecgonine	3	Femoral	358.0 ± 169.7	381.0	178.0 - 515.0	.793
		Iliac	350.7 ± 125.3	419.0	206.0 - 427.0	
		Inguinal	333.3 ± 118.9	401.0	196.0 - 403.0	
Buprenorphine	3	Femoral	2.7 ± 2.2	2.9	0.5 - 4.8	.067
		Iliac	2.3 ± 2.2	2.6	0.0 - 4.3	
		Inguinal	0.6 ± 0.5	0.8	0.0 - 0.9	
Carbamazepine	3	Femoral	3.3 ± 3.2	1.7	1.2 - 7.0	.423
Epoxide (mg/L)		Iliac	3.2 ± 2.8	2.2	1.0 - 6.4	
		Inguinal	4.3 ± 4.7	1.9	1.3 - 9.8	
Clonazepam	3	Femoral	5.8 ± 0.8	5.6	5.1 - 6.7	.655
		Iliac	6.0 ± 1.0	6.0	5.0 - 7.0	
		Inguinal	6.1 ± 0.5	6.0	5.7 - 6.7	

DRUG/METABOLITE	*N*	BLOOD	MEAN ± SD	MEDIAN	RANGE	*P*
Codeine	3	Femoral	73.8 ± 83.3	27.6	23.8 - 170.0	.434
		Iliac	60.3 ± 56.1	28.8	27.0 - 125.0	
		Inguinal	56.5 ± 57.6	25.5	21.1 - 123.0	
Meprobamate	3	Femoral	20.6 ± 24.2	10.6	3.1 - 48.3	.507
(mg/L)		Iliac	19.0 ± 20.9	10.5	3.7 - 42.9	
		Inguinal	18.4 ± 20.0	9.7	4.2 - 41.3	
Norvenlafaxine	3	Femoral	373.2 ± 306.7	352.0	77.6 - 690.0	.330
		Iliac	557.5 ± 556.3	425.0	79.4 - 1168.0	
		Inguinal	442.8 ± 397.3	422.0	56.3 -850.0	
Oxymorphone	3	Femoral	35.6 ± 12.6	32.0	25.2 - 49.6	.496
		Iliac	45.9 ± 49.5	19.8	14.8 - 103.0	
		Inguinal	25.3 ± 33.8	12.2	0.0 - 63.7	
Pseudoephedrine	3	Femoral	317.7 ± 253.4	222.0	126.0 - 605.0	.734
		Iliac	295.3 ± 208.8	188.0	162.0 - 536.0	
		Inguinal	301.3 ± 268.7	182.0	113.0 - 609.0	
Valproic Acid	3	Femoral	27.6 ± 4.1	26.9	23.8 - 32.0	.950
(mg/L)		Iliac	28.6 ± 5.4	30.3	22.5 - 32.9	
		Inguinal	27.9 ± 5.8	28.6	21.8 - 33.3	
Carbon Monoxide	2	Femoral	8.0 ± 2.6	9.8	6.1 - 9.8	.444
(% sat)		Iliac	2.9 ± 4.1	0.0	0.0 - 5.8	
		Inguinal	2.7 ± 3.7	0.0	0.0 - 5.3	
Levetiracetam	2	Femoral	339.3 ± 457.8	339.3	15.6 - 663.0	.588
(mg/L)		Iliac	307.4 ± 403.9	307.4	21.8 - 593.0	
		Inguinal	308.3 ± 405.5	308.3	21.6 - 595.0	
6-acetylmorphine	2	Femoral	26.3 ± 1.9	26.3	24.9 - 27.6	.617
		Iliac	12.8 ± 18.0	12.8	0.0 - 25.5	
		Inguinal	20.8 ± 5.9	20.8	16.6 - 24.9	
Lidocaine	2	Femoral	2.1 ± 0.5	2.1	1.7 - 2.4	.372
		Iliac	1.9 ± 0.1	1.9	1.8 - 1.9	
		Inguinal	1.0 ± 0.8	1.0	0.4 - 1.5	
Norbuprenorphine	2	Femoral	5.6 ± 4.6	5.6	2.3 - 8.8	.067
		Iliac	6.0 ± 5.3	6.0	2.2 - 9.7	
		Inguinal	2.9 ± 4.0	2.9	0.0 - 5.7	
Oxazepam	2	Femoral	131.7 ± 96.7	131.7	63.3 - 200.0	.421
		Iliac	109.9 ± 68.1	109.9	61.7 - 158.0	
		Inguinal	109.5 ± 72.8	109.5	58.0 - 161.0	
Trazodone	2	Femoral	0.4 ± 0.0	0.4	0.3 - 0.4	.524
		Iliac	0.2 ± 0.2	0.2	0.0 - 0.4	
		Inguinal	0.3 ± 0.0	0.3	0.3 - 0.3	
Warfarin	2	Femoral	0.5 ± 0.1	0.5	0.4 - 0.5	.250
(mg/L)		Iliac	0.7 ± 0.3	0.7	0.5 - 0.9	
		Inguinal	0.8 ± 0.4	0.8	0.6 - 1.1	

Table 2: Summary of drugs and/or metabolite concentrations (ng/mL) in only one specimen triplet. Femoral blood was collected by blind stick and shipped to the laboratory at ambient temperature for comprehensive toxicology panel. Iliac blood was collected by direct visualization and clamping the vein. Inguinal blood was collected by blind stick of the femoral vein. Iliac and Inguinal blood were shipped to the laboratory on dry ice for comprehensive toxicology panel.

DRUG/METABOLITE	FEMORAL	ILIAC	INGUINAL
Alpha-PVP*	401.0	502.0	480.0
Carisoprodol (mg/L)	13.0	10.5	9.1
Chlordiazepoxide	644.0	660.0	837.0
Clomipramine	224.0	422.0	448.0
Clozapine	725.0	818.0	729.0
Doxepin	5899.0	4527.0	3904.0
Doxylamine	324.0	205.0	166.0
Methylphenidate	54.8	44.0	28.9
Midazolam	77.1	0.0	0.0
Norclomipramine	708.0	2546.0	2403.0
Norclozapine	242.0	262.0	215.0
Nordoxepin	598.0	655.0	577.0
Rocuronium	1013.0	0.0	0.0
Venlafaxine	1180.0	1334.0	1383.0
Demoxepam	185.0	145.0	192.0
Hydroxyzine	65.2	59.9	31.4

*Alpha-pyrrolidinopentiophenone

There was large variability in drug concentrations within and between subjects. In 49 specimen triplets, the femoral blood specimen (Specimen 1) was positive while the iliac (Specimen 2), inguinal (Specimen 3) or both were negative (Table 3). This occurred for THC (*n = 9*), fluoxetine (*n = 4*), norsertraline (*n = 4*) hydromorphone (*n = 3*), norfluoxetine (*n = 2*), nortriptyline (*n = 2*), oxycodone (*n = 2*), promethazine (*n = 2*), sertraline (*n = 2*), and on a single specimen triplet for multiple other drugs (Table 3).

Table 3: Summary of drug/metabolite concentrations (ng/mL) found in only one or two of the specimens. Femoral blood was collected by blind stick and shipped to the laboratory at ambient temperature for comprehensive toxicology panel. Iliac blood was collected by direct visualization and clamping the vein. Inguinal blood was collected by blind stick of the femoral vein. Iliac and Inguinal blood were shipped to the laboratory on dry ice for comprehensive toxicology panel.

DRUG/METABOLITE	FEMORAL	ILIAC	INGUINAL
THC	12.6	3.7	0
THC	2.6	0	0
THC	1.8	0	0
THC	1.2	0	0
THC	1.8	3.1	0
THC	3.4	1.3	0
THC	1.2	0	0
THC	3.1	0	0
THC	8.6	2.5	0
Sertraline	135	0	0
Sertraline	201	0	139
Sertraline	1138	0	178
Sertraline	133	0	0
Norsertraline	351	0	0
Norsertraline	1027	360	0
Norsertraline	2750	0	179
Norsertraline	162	0	0
Fluoxetine	111	0	0
Fluoxetine	308	244	0
Fluoxetine	24.8	0	0
Fluoxetine	65.1	64.3	0
Hydromorphone	5.1	0	18.1
Hydromorphone	3.1	4.4	0
Hydromorphone	2.6	2.6	0
EDDP	32.4	25.4	0
EDDP	27.8	30.8	0
Norfluoxetine	281	39.5	0
Norfluoxetine	121	95.5	0
Nortriptyline	228	0	0
Nortriptyline	41.4	20.3	0
Oxycodone	15.1	14.4	0
Oxycodone	10.1	11.3	0
Promethazine	82.9	0	0
Promethazine	27.3	0	0
6-acetylmorphine	24.9	0	24.9
7-aminoclonazepam	12.5	0	18.5
Amitriptyline	23.3	20.3	0
Amlodipine	26.6	0	13.4
Amphetamine	65.4	66.4	0
Buprenorphine	0.5	0	0
Carbon Monoxide (% sat)	9.8	0	0
Dextromethorphan	16.2	25.8	0
Diazepam	59.8	0	0
Diphenhydramine	56.5	53.3	0
Duloxetine	133	57.6	0
Fentanyl	5.3	0	10.1
Midazolam	77.1	0	0

DRUG/METABOLITE	FEMORAL	ILIAC	INGUINAL
Tramadol	284	0	0
Trazodone	0.33	0	0.29

Within subject variability was characterized by calculating concentration ratios. We found large variability in iliac/femoral, inguinal/femoral and iliac/inguinal ratios, although concentrations between the three sites within a specimen triplet were generally very similar (Table 4). The mean ratios among all drug/metabolites were 1.0, 0.9 and 1.2 for iliac/femoral, inguinal/femoral and iliac/inguinal comparisons, respectively. The lowest ratio was 0.0 (one specimen was negative), found in multiple specimen triplets (see Table 3) and the highest ratio was 10.9 found when comparing inguinal/femoral blood positive for THC.

Table 4: Summary of drug/metabolite mean concentration ratios (range). Femoral blood (FEM) was collected by blind stick and shipped to the laboratory at ambient temperature for comprehensive toxicology panel. Iliac blood (ILIAC) was collected by direct visualization and clamping the vein. Inguinal blood (ING) was collected by blind stick of the femoral vein. Iliac and Inguinal blood were shipped to the laboratory on dry ice for comprehensive toxicology panel.

DRUG/METABOLITE	N	ILIAC/FEM	ING/FEM	ILIAC/ING
6-acetylmorphine	2	0.5 (0.0 - 0.9)	0.8 (0.6 - 1.0)	0.8 (0.0 - 1.5)
7-aminoclonazepam	21	1.6 (0.2 - 3.9)	1.8 (0.2 - 4.5)	1.0 (0.6 - 3.0)
Acetaminophen	8	1.1 (0.8 - 1.7)	0.9 (0.5 - 1.3)	1.2 (0.8 - 1.7)
Alpha-PVP	1	1.3 (1.3 - 1.3)	1.2 (1.2 - 1.2)	1.0 (1.0 - 1.0)
Alprazolam	37	1.0 (0.4 - 1.6)	1.1 (0.6 - 2.2)	1.0 (0.4 - 1.8)
Amitriptyline	9	0.9 (0.3 - 1.2)	0.8 (0.0 - 1.4)	1.5 (0.2 - 5.1)
Amlodipine	6	2.2 (0.0 - 8.8)	1.7 (0.5 - 6.1)	1.1 (0.0 - 1.9)
Amphetamine	5	1.2 (0.9 - 1.7)	0.7 (0.0 - 1.1)	1.6 (1.1 - 2.0)
Benzoylecgonine	3	1.0 (0.8 - 1.2)	1.0 (0.8 - 1.1)	1.1 (1.0 - 1.1)
Buprenorphine	3	0.6 (0.0 - 0.9)	0.2 (0.0 - 0.3)	4.1 (2.9 - 5.4)
Bupropion	4	1.0 (0.9 - 1.1)	1.1 (0.8 - 1.4)	1.0 (0.6 - 1.3)
Carbamazepine	5	1.0 (0.8 - 1.0)	1.0 (0.9 - 1.4)	1.0 (0.8 - 1.1)
Carbamazepine Epoxide	3	1.0 (0.8 - 1.3)	1.2 (1.1 - 1.4)	0.9 (0.7 - 1.2)
Carbon Monoxide	2	0.5 (0.0 - 1.0)	0.4 (0.0 - 0.9)	1.1 (1.1 - 1.1)
Carisoprodol	1	0.8 (0.8 - 0.8)	0.7 (0.7 - 0.7)	1.2 (1.2 - 1.2)
Chlordiazepoxide	1	1.0 (1.0 - 1.0)	1.3 (1.3 - 1.3)	0.8 (0.8 - 0.8)
Citalopram	14	2.1 (0.6 - 7.8)	1.2 (0.2 - 2.8)	1.6 (0.8 - 3.6)
Clomipramine	1	1.9 (1.9 - 1.9)	2.0 (2.0 - 2.0)	0.9 (0.9 - 0.9)
Clonazepam	3	1.0 (0.9 - 1.2)	1.1 (1.0 - 1.2)	1.0 (0.9 - 1.0)
Clozapine	1	1.1 (1.1 - 1.1)	1.0 (1.0 - 1.0)	1.1 (1.1 - 1.1)
Codeine	3	1.0 (0.7 - 1.2)	0.9 (0.7 - 1.1)	1.1 (1.0 - 1.3)
Cyclobenzaprine	6	0.9 (0.6 - 1.3)	1.2 (0.7 - 1.4)	0.8 (0.6 - 1.1)
Demoxepam	1	0.8 (0.8 - 0.8)	1.0 (1.0 - 1.0)	0.8 (0.8 - 0.8)
Dextromethorphan	4	1.3 (1.1 - 1.6)	0.8 (0.0 - 1.2)	1.2 (1.0 - 1.3)
Diazepam	10	0.9 (0.0 - 1.4)	1.0 (0.0 - 1.7)	0.9 (0.7 - 1.1)
Diphenhydramine	14	0.8 (0.4 - 1.5)	0.8 (0.0 - 1.1)	1.0 (0.3 - 1.5)

DRUG/METABOLITE	N	ILIAC/FEM	ING/FEM	ILIAC/ING
Doxepin	1	0.8 (0.8 - 0.8)	0.7 (0.7 - 0.7)	1.2 (1.2 - 1.2)
Doxylamine	1	0.6 (0.6 - 0.6)	0.5 (0.5 - 0.5)	1.2 (1.2 - 1.2)
Duloxetine	5	1.0 (0.4 - 1.4)	0.8 (0.0 - 1.2)	1.2 (0.9 - 1.7)
EDDP	9	1.0 (0.8 - 1.4)	0.6 (0.0 - 1.0)	1.3 (1.0 - 1.5)
Ethanol	15	1.0 (0.8 - 1.4)	1.0 (0.9 - 1.1)	1.0 (0.8 - 1.4)
Fentanyl	9	0.9 (0.0 - 1.3)	1.1 (0.7 - 1.9)	0.9 (0.0 - 1.7)
Fluoxetine	7	0.7 (0.0 - 1.1)	0.4 (0.0 - 1.5)	1.4 (0.7 - 2.8)
Gabapentin	8	1.1 (0.7 - 1.7)	1.1 (0.6 - 1.9)	1.1 (0.5 - 2.0)
Hydrocodone	12	1.2 (0.5 - 2.1)	0.9 (0.3 - 1.5)	1.4 (0.9 - 2.5)
Hydromorphone	7	0.9 (0.0 - 1.4)	1.2 (0.0 - 3.5)	0.7 (0.0 - 1.1)
Hydroxyzine	1	0.9 (0.9 - 0.9)	0.5 (0.5 - 0.5)	1.9 (1.9 - 1.9)
Lamotrigine	6	0.9 (0.6 - 1.3)	0.8 (0.4 - 1.0)	1.3 (0.7 - 2.4)
Levetiracetam	2	1.1 (0.9 - 1.4)	1.1 (0.9 - 1.4)	1.0 (1.0 - 1.0)
Lidocaine	2	0.9 (0.8 - 1.1)	0.5 (0.2 - 0.9)	2.9 (1.3 - 4.5)
Lorazepam	6	0.9 (0.6 -1.2)	0.8 (0.0 - 1.5)	1.0 (0.7 - 1.3)
Meprobamate	3	1.0 (0.9 -1.2)	1.0 (0.9 - 1.4)	1.0 (0.9 - 1.1)
Methadone	13	1.0 (0.5 - 1.8)	0.9 (0.6 - 1.5)	1.1 (0.5 - 1.7)
Methamphetamine	7	1.2 (0.9 - 1.5)	1.1 (0.5 - 1.8)	1.2 (0.6 - 1.9)
Methylphenidate	1	0.8 (0.8 - 0.8)	0.5 (0.5 - 0.5)	1.5 (1.5 - 1.5)
Metoprolol	6	1.1 (1.0 - 1.4)	1.1 (0.7 - 1.5)	1.1 (0.9 - 1.4)
Midazolam	1	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)
Mirtazapine	6	0.9 (0.5 - 1.4)	1.0 (0.4 - 1.3)	0.9 (0.6 - 1.1)
Morphine	19	1.2 (0.4 - 3.9)	1.1 (0.4 - 3.0)	1.5 (0.2 - 4.7)
Norbuprenorphine	2	1.0 (1.0 - 1.1)	0.3 (0.0 - 0.6)	1.4 (1.0 - 1.7)
Norclomipramine	1	3.6 (3.6 - 3.6)	3.4 (3.4 - 3.4)	1.1 (1.1 - 1.1)
Norclozapine	1	1.1 (1.1 - 1.1)	0.9 (0.9 - 0.9)	1.2 (1.2 - 1.2)
Nordiazepam	11	1.1 (0.7 - 1.7)	1.1 (0.7 - 1.9)	1.0 (0.7 - 1.2)
Nordoxepin	1	1.1 (1.1 - 1.1)	1.0 (1.0 - 1.0)	1.1 (1.1 - 1.1)
Norfluoxetine	6	0.7 (0.1 - 1.4)	0.6 (0.0 - 1.7)	2.8 (0.7 - 9.1)
Norsertraline	5	0.2 (0.0 - 0.5)	0.2 (0.0 - 0.8)	0.3 (0.0 - 0.7)
Nortramadol	8	1.3 (0.8 - 2.0)	1.3 (1.0 - 2.1)	1.0 (0.4 - 1.3)
Nortriptyline	10	0.7 (0.0 - 1.3)	0.6 (0.0 - 1.2)	1.3 (0.2 - 3.0)
Norvenlafaxine	3	1.3 (1.0 - 1.7)	1.1 (0.7 - 1.2)	1.3 (1.0 - 1.4)
Oxazepam	2	0.9 (0.8 - 1.0)	0.9 (0.8 - 0.9)	1.0 (1.0 - 1.1)
Oxycodone	16	1.1 (0.7 - 1.8)	0.9 (0.0 - 1.4)	1.1 (0.7 - 1.8)
Oxymorphone	3	1.1 (0.6 - 2.1)	0.6 (0.0 - 1.3)	1.4 (1.2 - 1.6)
Paroxetine	7	1.0 (0.8 - 1.2)	0.8 (0.3 - 1.2)	1.5 (0.7 - 3.6)
Pregabalin	6	1.0 (0.1 - 1.8)	1.0 (0.1 - 1.6)	1.0 (0.9 - 1.1)
Promethazine	3	0.3 (0.0 - 0.9)	0.3 (0.0 - 1.0)	0.9 (0.9 - 0.9)
Pseudoephedrine	3	1.0 (0.8 - 1.3)	0.9 (0.8 - 1.0)	1.1 (0.9 - 1.4)
Quetiapine	6	2.6 (0.9 - 9.2)	1.7 (0.7 - 4.1)	1.4 (0.9 - 2.2)
Rocuronium	1	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)
Sertraline	5	0.1 (0.0 - 0.5)	0.2 (0.0 - 0.7)	0.6 (0.0 - 1.7)
Temazepam	6	1.0 (0.8 - 1.2)	1.1 (0.8 - 2.3)	1.0 (0.4 - 1.3)
THC	14	0.6 (0.0 - 1.7)	1.1 (0.0 - 10.9)	0.8 (0.1 - 1.3)
THC-COOH	12	1.0 (0.5 - 2.2)	0.8 (0.4 - 2.0)	1.3 (0.7 - 2.2)
Tramadol	7	0.9 (0.0 - 1.3)	1.0 (0.0 - 1.9)	1.0 (0.6 - 1.3)
Trazodone	2	0.4 (0.0 - 0.9)	0.8 (0.7 - 0.9)	0.6 (0.0 - 1.3)
Valproic Acid	3	1.1 (0.8 - 1.4)	1.0 (0.8 - 1.2)	1.0 (0.9 - 1.2)
Venlafaxine	1	1.1 (1.1 - 1.1)	1.2 (1.2 - 1.2)	1.0 (1.0 - 1.0)
Warfarin	2	1.4 (1.1 - 1.6)	1.7 (1.4 - 2.0)	0.8 (0.8 - 0.8)
Zolpidem	6	1.0 (0.7 - 1.6)	0.8 (0.4 - 1.3)	1.4 (0.7 - 1.8)

4. CONCLUSIONS

4.1 Statistical Significance

Reliable interpretation of postmortem blood drug concentrations depends greatly on pre-analytical variables such as collection site and possible analyte instability. Blood collected from any peripheral site, regardless of collection method, is often considered the most reliable specimen when interpreting toxicological findings. We found no statistical difference between the three collection/shipping methods for most drugs. This finding is not surprising considering the small sample size within each drug/metabolite group and the large variability in drug concentration within each specimen collection/shipping procedure. For three analytes, however, statistical differences were detected. Methadone metabolite (2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine, EDDP), delta-9-carboxy-THC and amphetamine concentrations in the inguinal specimen were significantly lower than in the other two sites. Meaningful conclusions based on these findings remain suspect. While statistically significant differences were detected, small sample size should be considered. Nevertheless, one possible explanation may include analyte instability in inguinal blood after a single freeze/thaw cycle. The inguinal specimen was collected from approximately the same site and immediately following the femoral blood specimen. The only differences between these two collections is that, while taken from the same general location in the inguinal region, each was obtained by a different blind stick of the femoral vein. The femoral blood (Specimen 1) was temporarily refrigerated prior to submission to the laboratory and was shipped at ambient temperature shortly after autopsy. The inguinal specimen (Specimen 3), was immediately frozen at -57°C. Several weeks passed in some cases before informed consent was obtained from next-of-kin. Laboratory results on the femoral specimen were received within approximately week. No significant decrease in EDDP

concentration was found in -20°C stored breast milk after one month (Nikolaou, Papoutsis et al. 2008); however, EDDP was found to be unstable in oral fluid stored refrigerated for two months (Fucci and De Giovanni 2008). Conversion of methadone to EDDP occurs by demethylation *in vivo* (Danielson, Mozayani et al. 2008); however, we are unaware of studies demonstrating this conversion spontaneously in authentic specimens or fortified samples.

Delta-9-carboxy-THC concentrations may change when stored at various conditions after collection. In a recent study, whole blood specimens were collected from cannabis users after controlled drug administration and pooled (Scheidweiler, Schwope et al. 2013). Samples were stored at room temperature, refrigerated (4°C) and frozen (-20°C) for various durations. Delta-9-carboxy-THC concentrations increased when stored at room temperature and remained stable for 4 an 26 weeks when stored refrigerated and frozen, respectively. This may be explained by conversion of delta-9-carboxy-THC-glucuronide to delta-9-carboxy-THC at higher temperature, which also was demonstrated in authentic urine specimens (Skopp and Potsch 2004). The authors also evaluated delta-9-carboxy-THC-glucuronide instability in fortified samples at various pH. In that study, the magnitude of decrease and simultaneous increase in delta-9-carboxy-THC was pH dependent and occurred even at pH 5. Therefore, our data appear to be consistent with an increase in delta-9-caboxy-THC when collected in the femoral vein and shipped at ambient temperature, instead of its decrease in the other two specimens maintained frozen.

As stated above, statistical conclusions based on a small sample size remain suspect; however, significantly lower amphetamine concentration in the inguinal specimen may be explained by site of collection and/or PMR. Amphetamine appears to be stable at room temperature, with moderate decreases over three months (Giorgi and Meeker 1995). The short period in which the femoral blood was shipped at ambient temperature should not cause

significant amphetamine concentration decreases. Post Hoc analysis revealed that the inguinal blood amphetamine concentration was significantly lower than the iliac blood concentration. Both of these specimens were collected and shipped to the laboratory after frozen storage. Given its relative stability, this finding suggests possible PMR into iliac site. The iliac vein found within the abdominal cavity and some drugs may re-distribute into the iliac blood to a greater extent than more distal sites such as femoral blood. Subclavian blood was recently evaluated to determine whether drug concentrations were closer to peripheral or heart blood concentrations (Molina and Hargrove 2013). The authors found that generally, drug concentrations were lower in subclavian blood than in heart blood, but higher than in peripheral blood and recommended the specific site be included in postmortem toxicology evaluation.

Trends toward significance ($p < 0.10$) were determined for nortiptyline ($n = 10$, $p = 0.065$), N-desmethyltramadol ($n = 8$, $p = 0.074$), buprenorphine ($n = 3$, $p = 0.067$) and norbuprenorphine ($n = 2$, $p = 0.067$). As with the aforementioned analytes, different drug concentrations for nortriptyline, N-desmtehyltramadol, buprenorphine and norbuprenorphine, may be explained by analyte instability or potential PMR within the peripheral compartment. Nortriptyline appears to be stable in plasma (Hotha, Ravindranath et al. 2010) and should not significantly degrade at ambient or frozen temperature. Buprenorphine and norbuprenorphine in whole blood also appear to be stable at -20°C (Seldén, Roman et al. 2011). To our knowledge, postmortem conversion from amitriptyline to nortriptyline or buprenorphine to norbuprenorphine has not been shown. These data demonstrate potential site dependent or pre-analytical changes in drug concentrations for these analytes.

4.2 Postmortem redistribution

Postmortem redistribution of drugs is widely published in the literature. Several reviews have been published addressing topics such as interpretation of drug levels and pharmacokinetic relationships (Ferner 2008), the relationship between the putrefactive process and movement of drugs into different compartments (Pelissier-Alicot, Gaulier et al. 2003) and estimating ante-mortem drug concentrations from autopsy specimens (Cook, Braithwaite et al. 2000). Multiple others have investigated PMR of individual drugs. The mechanisms of PMR are complex and involve movement of drugs along concentration gradients from various organs, changes in blood properties such as putrefaction and multiple chemical properties of the individual drug, such as pK_a and lipophilicity (Pelissier-Alicot, Gaulier et al. 2003). Additionally, putrefaction and the influence of bacteria may alter drug concentrations in the postmortem interval or after specimen collection (Butzbach 2010). These reviews help to explain the complexity of PMR and the difficulty it poses in drug related deaths.

We found multiple specimen triplets in which iliac or inguinal or both blood specimens were negative. Cases were selected based in part on whether femoral blood (Specimen 1) was positive for one or more drugs/metabolites. Drug concentrations in the remaining two specimens were assumed to be positive, at least at the time of autopsy. Negative findings in these specimens suggests potential degradation of analytes in the frozen specimens (Specimens 2 and 3), or artificially elevated concentration in the initial specimen, owing to PMR. Given the large variability in analyte concentrations, it also is possible that some drugs/metabolites were below the analytical cutoff in one or two specimens due to an overall low venous blood concentration. Low analyte concentrations near the cutoff were found for THC, hydromorphone and buprenorphine (see Table 3); however, the remaining analytes listed in Table 3 were well within

the detectable range. THC concentrations decreased significantly in the two frozen specimens, likely owing to adherence to container surfaces. This phenomenon has been documented previously with THC losses of 60 to 100% when specimens were stored in polystyrene tubes for one month (Christophersen 1986).

Another explanation may be PMR. The classical method of characterizing PMR is by comparing heart/peripheral blood drug/metabolite concentrations. A heart/peripheral blood ratio greater or less than 1 suggests potential re-distribution of drugs within these sites. Peripheral blood drug/metabolite concentration is believed to be more stable after death than heart blood due to re-distribution from multiple potential tissue sites within the central cavity. Few studies have examined potential re-distribution within the peripheral compartment. One study demonstrated re-distribution of fentanyl by comparing fentanyl concentrations in femoral blood collected shortly after death to a second specimen collected at autopsy (Olson, Luckenbill et al.). The mean concentrations for the first and second femoral blood specimens were 4.6 ng/mL to 17.6 ng/mL, respectively. In all cases, the concentration increased in the second specimen.

We examined concentration ratios between the three specimens to demonstrate within subject variability in analyte concentration (Table 4). Generally, concentrations were similar between the three specimens; however, some ratios were less than 0.5 or greater than 2.0. This indicates that pre-analytical factors influence drug concentration and potentially toxicological interpretation. Therefore, while generally it appears that any of these collection/shipping procedures should yield similar results, there still is the potential for misinterpretation based on peripheral blood concentration. For example, an increase in concentration due to PMR, from within the therapeutic to within the toxic range, could yield an incorrect interpretation of toxicity.

Usually, multiple specimens are collected during autopsy for toxicological determination. Heart blood, peripheral blood, urine, vitreous fluid, liver and other organ tissues may be collected. Drug/metabolite concentrations in peripheral blood remain an essential part of the overall toxicological investigation; however, concentrations in other specimen types also aid interpretation. These data further support the practice of basing interpretation on the totality of evidence, including examination of peripheral blood and other specimen types, as well as other investigative factors.

To our knowledge, there are no other prospective studies that have compared drug/metabolite concentrations in multiple peripheral blood sites. The total scope of this study, number of different drugs/metabolites detected and total cases has not been reported elsewhere. These data will be valuable for toxicologists, pathologists and clinicians in interpreting drug/metabolite concentrations amid other case findings.

5. STUDY LIMITATIONS

The study was designed with parameters that were very specific in an effort to maximize the value of the data generated; however, these data are limited and should be applied only to applicable situations. Unlike many studies dealing with PMR, we did not compare differences in drug concentrations between peripherally and centrally obtained blood specimens. Instead, we concentrated solely on possible differences in analyte concentrations in peripheral sites, limited to femoral and iliac venous blood specimens, which are commonly collected during autopsy. PMR is an accepted phenomenon and studies are found widely throughout the literature. While heart blood was collected as part of the routine autopsy examination, this investigation evaluated the potential for PMR only within the peripheral compartment.

It is also important to note that all specimens were collected prior to evisceration. Therefore, the removal of blood from an unclamped vessel could draw from the central compartment via the inferior vena cava. In this study, we collected two specimens (Specimen 1 and Specimen 3) that were not ligated prior to collection. As previously described, this approach allowed the comparison of blood drug/metabolite concentrations using three commonly employed collection/shipping procedures. Specimens 1 and 3 differed only in their storage and shipping procedures. Therefore, this provided a useful comparison for evaluating analyte stability under these two conditions. Specimen volumes were relatively small (2.0 – 10.0 mL). Nevertheless, the potential contribution of blood drawn from Specimens 1 and 3 via communication with the central compartment may compromise this comparison. Also, we limited the study by only including specimens from decedents who had a relatively short time interval of death and did not include specimens from decedents who had time of death intervals longer than 48 hours based on the investigation of the circumstances of death or who were decomposed. Evaluating drug concentrations after longer postmortem duration offers valuable information, but has been published elsewhere and is beyond the scope of this investigation.

Another significant limitation of this study was that specimens were not split prior to submission for toxicological determination. The ideal statistical design for this study would involve splitting each specimen after collection and submitting one at ambient temperature and the other on dry ice. This would allow for the appropriate statistical comparisons between collection sites isolated as a single variable as well as shipping conditions as a different and potentially co-dependent variable. Instead, we compared three collection/shipping procedures where the collection and shipping method were combined into a single independent variable. We adopted this study design largely due to limited funding to support a large enough sample size

for each drug/metabolite or drug class. With this approach we were able to achieve enough statistical power to detect significant differences between the three specimen collection/shipping procedures for three drugs/metabolites. Additionally, this approach resulted in a large number of different analytes detected.

Difficulty in obtaining informed consent was an unanticipated limitation that was time consuming and resulted in specimens being stored frozen for extended periods. Informed consent protocol required an inordinate amount of time tracking down phone contacts. Often, phones were out of service, calls were never answered, next-of-kin could not be verified, or permission of inclusion into the study was refused. Despite our efforts to insure that no perceived rights had been ignored, the insistence of obtaining a valid informed consent became a setback in achieving the desired number of cases in this study.

Evaluation of analyte stability was not included as a primary objective for this study. Data on drug stability are widely published and include various experiments to define drug/metabolite stability in a number of contexts. The specific statistical design of this study did not allow for characterization of analyte stability, but rather provided a means of comparing concentrations in the three peripheral specimens. Analyte instability is only one potential explanation for the concentration differences detected in the three specimens.

A modification of the original grant submission involved eliminating the analysis of the effect that massaging the leg to obtain specimen may have impacted the results. Objective measurement of the degree of massaging/milking the leg was difficult to establish.

8. DISSEMINATION OF INFORMATION

These findings will be presented as either poster or platform presentations at the American Academy of Forensic Sciences (AAFS). Accepted presentations at this conference will be submitted as a manuscript(s) to AAFS' associated journal, The Journal of Forensic Sciences. Further manscript(s) discussing other aspects of this research not presented at AAFS may be submitted to other appropriate toxicology journals.

9. REFERENCES

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