JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, May 2011, Vol. 56, No. 3
doi: 10.1111/j.1556-4029.2011.01725.x
Available online at: onlinelibrary.wiley.com

PAPER

TOXICOLOGY

John F. Wyman,[1,2,†] Ph.D.; Dorothy E. Dean,[2] M.D.; Rachel Yinger,[1] B.S.; Amber Simmons,[1] M.S.; David Brobst,[1] B.S.; Michael Bissell,[2] M.D., Ph.D.; Fernando Silveira,[3] D.V.M.; Nancy Kelly[1]; Robert Shott,[2] M.D.; Joseph Ohr,[1] M.D.; Rick Howard,[3] B.S.; and Bradley Lewis,[1] M.D.

The Temporal Fate of Drugs in Decomposing Porcine Tissue*

ABSTRACT: Drug levels in decomposed individuals are difficult to interpret. Concentrations of 16 drugs were monitored in tissues (blood, brain, liver, kidney, muscle, and soil) from decomposing pigs for 1 week. Pigs were divided into groups ($n = 5$) with each group receiving four drugs. Drug cocktails were prepared from pharmaceutical formulations. Intracardiac pentobarbital sacrifice was 4 h after dosing, with tissue collection at 4, 24, 48, 96, and 168 h postdosing. Samples were frozen until assay. Detection and quantitation of drugs were through solid phase extraction followed by gas chromatograph/mass spectrometer analysis. Brain and kidneys were not available after 48 h; liver and muscle persisted for 1 week. Concentration of drugs increased during decomposition. During 1 week of decomposition, muscle showed average levels increasing but concentrations in liver were increased many fold, compared to muscle. Attempting to interpret drug levels in decomposed bodies may lead to incorrect conclusions about cause and manner of death.

KEYWORDS: forensic science, drugs, decomposing tissue, pig, liver, muscle, blood

The distribution of drugs immediately following death (the early postmortem period) and the phenomenon of postmortem redistribution have been well and extensively characterized (1–6). However, postmortem fluids that are routinely collected and analyzed (blood, urine, vitreous humor), and therefore provide the largest comparative database for interpretation, are lost as the postmortem interval increases. When bodies have decomposed to the point where no fluids are retrievable, the only specimens available are solid organs and bone. When drugs are found in weathered tissues, there is currently limited information available to help guide the toxicologist, and subsequently the pathologist, in evaluating whether a drug(s) played any significant role in causing the death (1,7,8). The lack of information about the fate of drugs in decomposing tissue is understandable, in that postmortem studies in humans are not a realistic possibility. This study used pigs as a pharmacological analog to humans and investigated the change in tissue drug levels as decomposition progresses.

Decomposing bodies are found in all types of environments, in and out of doors. The design of this study was to monitor concentrations of various drugs, in multiple tissues and organs, during whole body decomposition in the outside environment. The fate of sixteen drugs was followed in seven different tissues collected from decomposing pigs. Specific questions to be answered were:

- How do the concentrations of drugs change during tissue decomposition?
- How long do drugs and metabolites persist in tissues at detectable levels?
- Which collection sites are most useful for detecting drug exposure and interpreting results?

Materials and Methods

Animal Model

A suitable model for this study was *Sus domestica*, the domestic pig. Pigs were selected because their size is comparable to humans, which allowed high-level dosing and sequential sampling of multiple tissues. Also, their similar physiology to that of humans, specifically their digestive and cardiovascular physiology (9), allowed for drug absorption and distribution which closely mimic that occurring in humans. The pig has been used previously to study postmortem changes in the concentration of clozapine and norclozapine in blood and tissue (8). The animals used in this study were a Yorkshire/Hampshire cross-breed, females and/or male castrates (as available) and weighed approximately 120–180 pounds (55–82 kg). Pigs were obtained from Kidron Auction, Kidron, OH. The sample size was five animals each/four drug groups, with four negative controls. To administer drugs (described below), pigs were restrained using a nose snare, and with the aid of a speculum, drug

[1]Franklin County Coroner's Office, 520 King Ave, Columbus, OH 43201.
[2]Department of Pathology, School of Medicine, The Ohio State University, 4173 Graves Hall, 3333 10th Ave, Columbus, OH 43210.
[3]Department of Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210.
[†]Present Address: Cuyahoga County Regional Forensic Science Laboratory, Cuyahoga County Medical Examiner, 11001 Cedar Ave, Cleveland, OH 44106.
*This research was funded by a grant from the Midwest Forensics Resource Center, Ames, IA.

Received 2 Sept. 2009; and in revised form 2 Feb. 2010; accepted 2 April 2010.

cocktails were placed in the stomach by gavage using a 500-mL syringe and gastric tube.

Study Site

The animals were housed, prior to dosing, at the Finley Farm, The Ohio State University, West Jefferson, OH. This study was performed during the summer and early fall of 2007. The average daily temperature for decomposition of the four groups (A through D) is shown in Fig. 1.

Independent dosing of pigs was staggered over time to allow sufficient time (1 h per drug group) for sacrifice and transfer of animals to the study site. The dead animals were placed inside the perimeter of a chain-link fence, installed so that it was 2 feet below ground and 6 feet above ground, which served as a barrier to predators. Inside the chain-link fence, each carcass was housed in a rectangular protective cage, which was built from untreated 2×4 lumber and hardware cloth fencing. The hardware cloth fencing (six sides) prevented scavengers (birds and mice) from feasting on the carrion, but was sufficiently open to allow access for microorganisms, flies, and insects. Each cage held two carcasses.

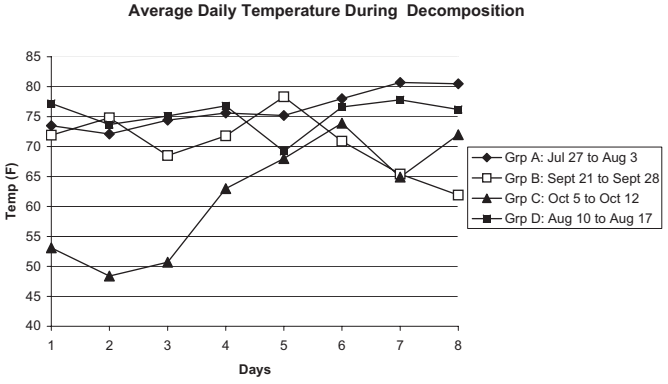

FIG. 1—*Environmental temperature during study period. (Data obtained form The Old Farmer's Almanac, http://www.almanac.com/weatherhistory.)*

Drugs Evaluated

Although the number of drugs which could be evaluated is vast, resources and time required the study to be limited in scope. Selection of the drugs to be evaluated was based on chemical stability during putrefaction, the prevalence of use of the drug in the general population, and the drug's propensity for causing or contributing to death in humans. Using these selection criteria, in essence, leaves other important parameters, such as volume of distribution and half-life, randomized. The stability of drugs in putrefying liver has been evaluated (10), and three structural criteria were found to contribute to molecular destruction: "the possession of a readily available oxygen for anaerobic purposes; the presence of suitably bonded sulphur; and an aminophenol structure where OH and NH_2 are present on the same aryl nucleus." The drugs listed in the following paragraph either were shown to be stable in the putrefaction study (10) or do not possess the functional groups found to make a molecule labile.

Drug cocktails were prepared from pills (capsules and tablets) available as a pharmaceutical formulation. The pills were dissolved/emulsified by sonication in water (total volume = 500 mL). An opioid analgesic was included as a component of each drug group to offset pain or discomfort which might be experienced by the animals. The concentration of drugs was prepared to achieve an expected toxic level [for humans (11,12)]. A toxic level would provide analgesia and sedation and allow for the highest chance of detection of drugs and metabolites in extracted tissues. Four drugs were administered to a single animal. Table 1 lists the specific drugs selected (groups A through D), the drug class, toxic concentration, and dosing amount for a 150 lb animal. Each group consisted of five pigs.

Tissue Collection

This study was performed from late spring to mid-autumn. Tissue collection times were approximately 4 h after dosing and then at 24, 48, 96, and 168 h. A 4-h delay prior to sacrifice allowed for partial gastric emptying, absorption of drugs, tissue distribution,

TABLE 1—*Drugs, toxic concentration, and dosing.*

Group	Class	Drug	V_D (11)	Toxic Conc., mg/L (11,12)	Dose/150 lb Pig (mg)†
A	Opioid	Morphine	3–5	>0.2	54.4
	TCA	Amitriptyline	15	>5	5100
	SSRI	Citalopram	12–16	>0.5	476
	BDZ	Diazepam	0.5–2.5	>5.0	510
					Total: 6140.4
B	Opioid	Methadone	4	>0.5	136
	SSRI	Fluoxetine	27	>1.0	1836
	TCA	Doxepin	20–24	>2.0	2992
	NSAID	Acetaminophen	1	>100	6800
					Total: 11,764
C	Opioid	Propoxyphene	16	>1.0	1088
	Antipsychotic	Olanzapine	22	>1.0	1496
	Antihistamine	Diphenhydramine	4.5–8	>5.0	2040
	SSRI	Venlafaxine	4–12	>7.0	3808
					Total: 8432
D	Opioid	Oxycodone	1.8–3.7	>0.4	76.2
	Muscle Relaxant	Carisoprodol	4*	>40	10880
	α-Blocker	Verapamil	2–6	>1.0	272
	Hypnotic	Zolpidem	0.5–0.7	>0.3	12.2
					Total: 11,240.4

TCA, tricyclic antidepressant; SSRI, serotonin selective reuptake inhibitor; BDZ, benzodiazepine; V_D, volume of distribution (L/Kg).

*No value reported for carisoprodal; the V_D for metoprolol, rather than meprobamate, the primary metabolite of carisoprodol, was mistakenly substituted.

†The amount (mg) of each drug to be administered was calculated as Amount = Toxic Conc*Body Wt*V_D; Body wt = 68 kg, Toxic Conc. = minimum value listed, and V_D was the average of the value listed (11).

and incomplete metabolism. Animals were anesthetized (30 mg/kg pentobarbital IP) and then sacrificed when they were sedated by intracardiac injection of 10 mL Beuthanasia-D® ([390 mg sodium pentobarbital sodium and sodium phenytoin, 390 and 50 mg/mL, resp.], Schering-Plough Animal Health, Union, NJ) solution using an 18G × 3 spinal needle (BD Biosciences, Bedford, MA). Animals were transferred to the study site, and necropsy performed in the field. Blood (5 mL or as much as possible) was collected from peripheral (iliac) and central sites (heart) and placed in 10 mL, gray top Vacutainer® collection tube (BD Medical Systems). Blood collection was a single collection at the time of sacrifice. Organs were removed, weighed, a small section of tissue excised (10–15 g), and then organs were returned to their anatomic location within the carcass for sampling at later time points. Specific tissues collected at each time period, or as long as available, included liver, brain, kidney, muscle, and maggots (13). Sampling of the liver was from the inferior border of the right lobe, with each sequential section removed adjacent to the previous collection site. Repetitive collection from adjacent sites reduced the potential variability that may have existed if multiple liver sites were selected. During collection of specimens, care was taken to avoid contact with the gall bladder, and excised sections were rinsed with water after collection to remove any bile from the surface of the tissue. Muscle tissue specimens were taken from the gluteus medius muscle of the hind limb. Repetitive samples were taken from the same incision site. Soil beneath the central compartment of the carcass was collected after 1 week. All specimens were placed in tubes or plastic specimen jars, as appropriate, returned to the laboratory, and frozen until analysis. The necropsy incisions were sutured closed and sealed with cyanoacrylate glue for the first three collection periods. To measure how necropsy affected total organ weight over time, 10 pigs, which were not treated, were sacrificed as before and dissected at each subsequent tissue collection point ($n = 2$) up to 48 h; $n = 1$ for 96 and 168 h. Dried liver specimens were prepared using a CentriVap® cold-trap centrifuge (Genetic Research Instrumentation Ltd, Braintree, Essex, U.K.).

Extraction of Drugs from Tissues

All chemicals used in the analyses were of reagent grade quality or better. Standards used to prepare calibrators were obtained from Cerilliant (Round Rock, TX). Control drugs prepared in whole blood were obtained from UTAK Laboratories, Inc., Valencia, CA. Internal standards included in extractions were SKF-525A (Calbiochem, San Diego, CA), hexobarbital and nalorphine (both obtained from Cerilliant). Procedures used to extract and analyze drugs were those which are routinely performed at Franklin County Coroner's Office for postmortem toxicology. Tissue specimens were thawed, an aliquot removed, and, except for blood, weighed, and homogenized (Waring® blender) in saline (brain = 1:1 dilution and other tissues = 1:5 dilution). Internal standards (SKF525A, hexobarbital, and/or nalorphine) were added to blood and homogenates (2 mL) prior to extraction. Drugs were extracted from tissues using solid phase extraction columns (CSDAU 206; United Chemical Technologies, Bristol, PA) (14). Two milliliters of specimens containing internal standards were diluted with distilled water (3 mL), adjusted to pH 6 with 1.0 M phosphate buffer (2 mL) and centrifuged. Supernatants were added to SPE columns which had been previously conditioned. Both basic and neutral drugs were extracted on to the sorbent bed. Neutral drugs were eluted with 3 mL hexane/ethylacetate (1:1); basic drugs were eluted with 3 mL methylene chloride/isopropyl alcohol/ammonium hydroxide (78:20:2). Eluents were dried under nitrogen and resuspended in 50 μL acetonitrile. Analysis of basic/acidic/neutral drugs and opiates were performed using gas chromatograph/mass spectrometer (GC/MS) or GC/MS with selective ion monitoring (SIM); quantitation employed certified calibrators (Cerilliant) and commercially prepared, custom controls (UTAK Laboratories, Inc.).

GC-MS Analysis

Chromatographic analysis was performed using an HP 5890 series II GC with an HP 5972 MS and HP 7673 autoinjector. The MS was operated in scan mode for all drugs except morphine. Morphine was derivatized with heptafluorbutyric anhydride and assayed by SIM: morphine: 464 and 480 *m/z*; nalorphine 490 *m/z*. Chromatographic conditions for basic/neutral/acidic drugs and morphine were the same except where indicated: Carrier gas: helium (1 mL/min); column: J&W Scientific HP-1 (cross-linked 100% dimethylpolysiloxane), 12 m, 0.200 mm i.d., 0.33-mm film thickness; column oven temperature program: 140°C (160°C for morphine) (1 min) to 290°C (3 min) increasing at a rate of 20°C/min; total run time = 11.5 min (8 min for morphine). Splitless injection, temperature = 250°C; detector temperature = 300°C; MS ionization energy was operated 70 eV, and mass spectra were collected by scanning from 43 to 550 *m/z*, at 2 s intervals (SIM for morphine). All analytes were linear to 2.0 μg/mL (1.2 μg/mL for morphine), and limits of quantitation were 0.1 μg/mL (0.08 μg/mL for

TABLE 2—*Concentration of drugs in iliac blood and tissues along with blood to tissue ratios.**

Drug	[Blood]	Relative to Human Conc	[Liver]	Blood/ Liver Ratio	[Muscle]	Blood/ Muscle Ratio	[Brain]	Blood/ Brain Ratio	[Kidney]	Blood/ Kidney Ratio
Amitriptyline	0.80 ± 0.39	T	14 ± 3.0	0.05	2.1 ± 1.4	0.39	5.4 ± 1.6	0.15	2.3 ± 0.54	0.35
Citalopram	0.53 ± 0.27	T	5.1 ± 0.90	0.10	1.4 ± 0.29	0.38	10 ± 3.6	0.05	3.1 ± 0.85	0.17
Diazepam	0.13 ± 0.06	T	4.7 ± 1.8	0.03	0.37 ± 0.08	0.35	1.1 ± 0.27	0.12	0.90 ± 0.17	0.14
Doxepin	3.6 ± 0.47	X–L	67 ± 16	0.05	5.9 ± 2.1	0.63	7.12 ± 2.1	0.52	24 ± 6.0	0.15
Fluoxetine	0.39 ± 0.17	T	16 ± 4.0	0.02	1.8 ± 0.63	0.22	6.5 ± 2.4	0.06	7.3 ± 2.2	0.05
Methadone	0.20 ± 0.05	T–X	2.6 ± 1.4	0.08	0.94 ± 0.12	0.21	0.74 ± .3	0.27	2.0 ± 0.37	0.10
Diphenhydramine	2.1 ± 0.74	T–X	33 ± 13	0.06	11.7 ± 0.84	0.18	6.7 ± 1.6	0.31	15 ± 7.0	0.14
Morphine	Trace	(<0.05)								
Propoxyphine	0.95 ± 0.35	T	13 ± 4.9	0.07	1.9 ± 0.54	0.49	2.3 ± 0.7	0.41	5.3 ± 2.0	0.18
Venlafaxine	7.1 ± 2.3	T–X	67 ± 23	0.11	48 ± 2.8	0.15	55 ± 10	0.13	58 ± 23	0.12
Carisoprodol	19 ± 5.6	T	21 ± 3.9	0.94	13 ± 2.7	1.56	34 ± 15	0.57	20 ± 5.0	0.96
Acetaminophen	15 ± 2.4	T	5.6 ± 0.55	2.8	22 ± 3.7	0.71	7.2 ± 0.6	2.2	87 ± 15	0.18

*Values are microgram per milliliter for blood and microgram per gram for tissue (mean ± standard error of the mean, $n = 5$) and represent concentrations at the time of sacrifice of pigs (T_0). The blood levels are compared to human blood levels (11,12) with T = therapeutic, X = toxic, L = lethal concentrations.

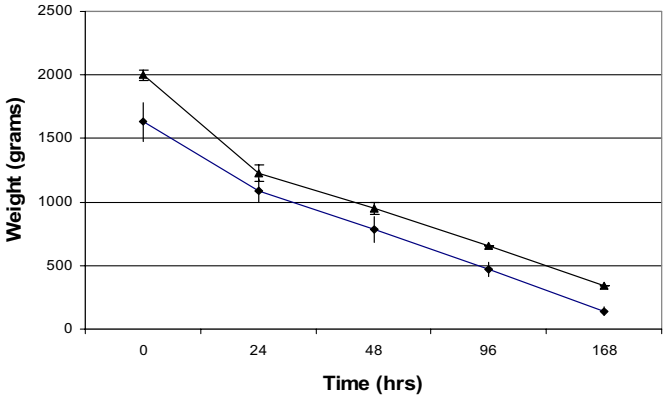

FIG. 2—*Loss of liver weight over time in necropsied and non-necropsied pigs.* ▲ *Liver weight in Control (non-necropsied) pigs;* n = *2 for 0–48 h.* ◆ *Liver weight in necropsied pigs;* n = *5.*

morphine). Acetaminophen was analyzed by fluorescence polarization immunoassay using an Abbott TDx® (Abbott Laboratories, Abbott Park, IL).

Statistical Evaluation

The sample size (*n*) was dictated by practical logistical considerations, and preliminary power calculations were therefore not made. The drug concentration results were analyzed by drug and anatomic site over time. One-way analysis of variance (ANOVA) was utilized on Minitab statistical software, release 13, Windows 98 version (Minitab Inc., State College, PA, 2000). Statistical significance of trends is indicated by the *p* value for *F* statistic in each case.

Results and Discussion

Table 2 depicts the average drug concentration in peripheral blood and tissues immediately following sacrifice of the pigs. Interpretation of these levels in terms of human drugs concentrations (i.e., therapeutic, toxic, lethal) is included in this table, along with blood tissue ratios. Only 11 of the 15 drugs were detected in the initial blood draw. A possible explanation why zolpidem, oxycodone, and verapamil were not detected in initial blood draws may be the relatively small amounts that were administered, compared to the other drugs (Table 1). As a result, these drugs may have been more rapidly cleared than the other drugs or diluted below detection because of distribution.

Average blood/tissue ratios for the initial specimen collection show drugs to be more concentrated in tissue than in peripheral blood, with the exception of acetaminophen in liver and brain and carisoprodol in muscle. Contrary to reports in the literature (15–17), these data also indicate that none of the tissues collected would be uniformly useful for predicting blood concentration of drugs.

Determining whether drug concentration in blood increased with increasing decomposition was not possible because of the initial disruption of the iliac vein; a second blood draw could not be made. It is well established that drug concentrations in postmortem blood differ from those observed in antemortem specimens, especially if the drug undergoes postmortem redistribution (1–6). However, the focus of this study was on what happens to the concentration of drugs in tissue, when blood is not available for collection.

In the summer time, in an outside environment, pig carcasses decomposed rapidly. After 2 days, tissues not available for analysis

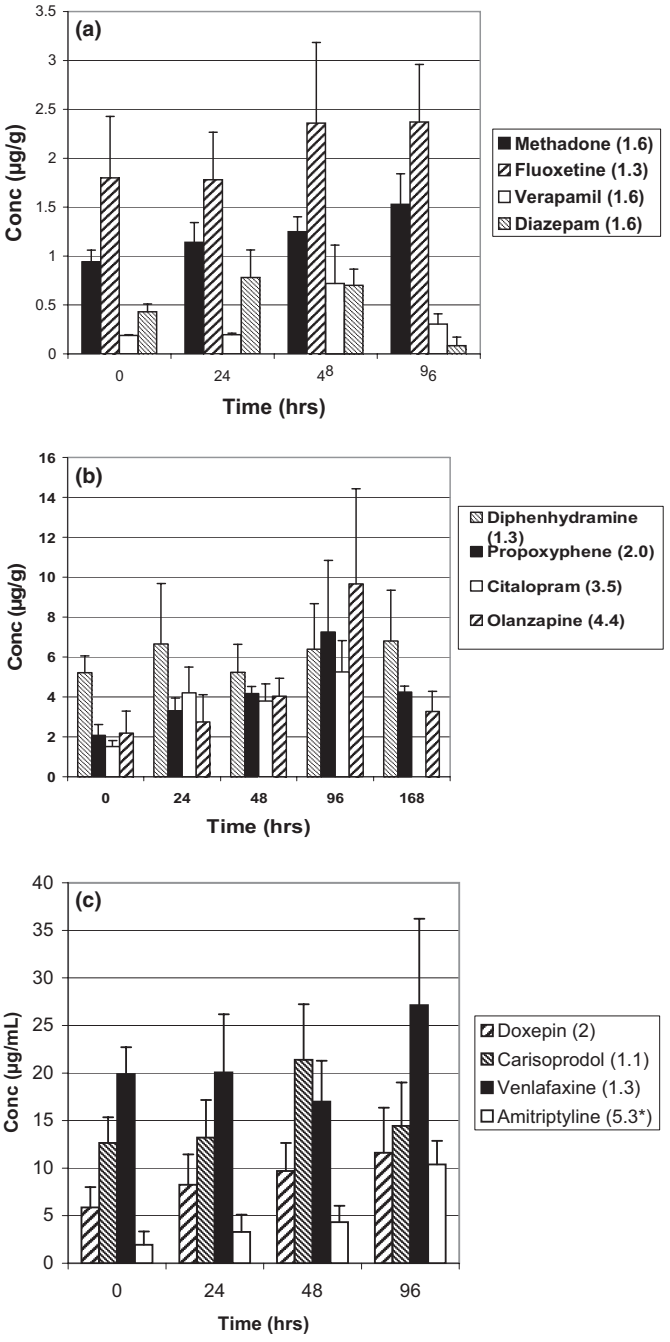

FIG. 3—*(a) Average temporal increase in methadone, fluoxetine, verapamil, and diazepam in decomposing muscle; error bars represent SEM. (b) Average temporal increase in diphenhydramine, propoxyphene, citalopram, and olanzapine in decomposing muscle; error bars represent SEM. (c) Average temporal increase in doxepin, carisoprodal, venlafaxine, and amitriptyline in decomposing muscle; error bars represent SEM, and statistical significance is indicated by *.*

included blood, brain, vitreous, and kidney. Analysis of maggots collected from carcasses demonstrated the presence of drugs as early as 48 h (diazepam, citalopram, amitriptyline). All drugs analyzed were present in maggots collected after 96 h. The tissues that persisted for 1 week were liver and muscle. Drugs were present in soil, collected at 1 week, from beneath the abdomen of carcasses. (After the preparation of this manuscript, soil was collected from the site where a pig dosed with amitriptyline, citalopram, diazepam, and morphine had decomposed. Drugs persisting in soil more than

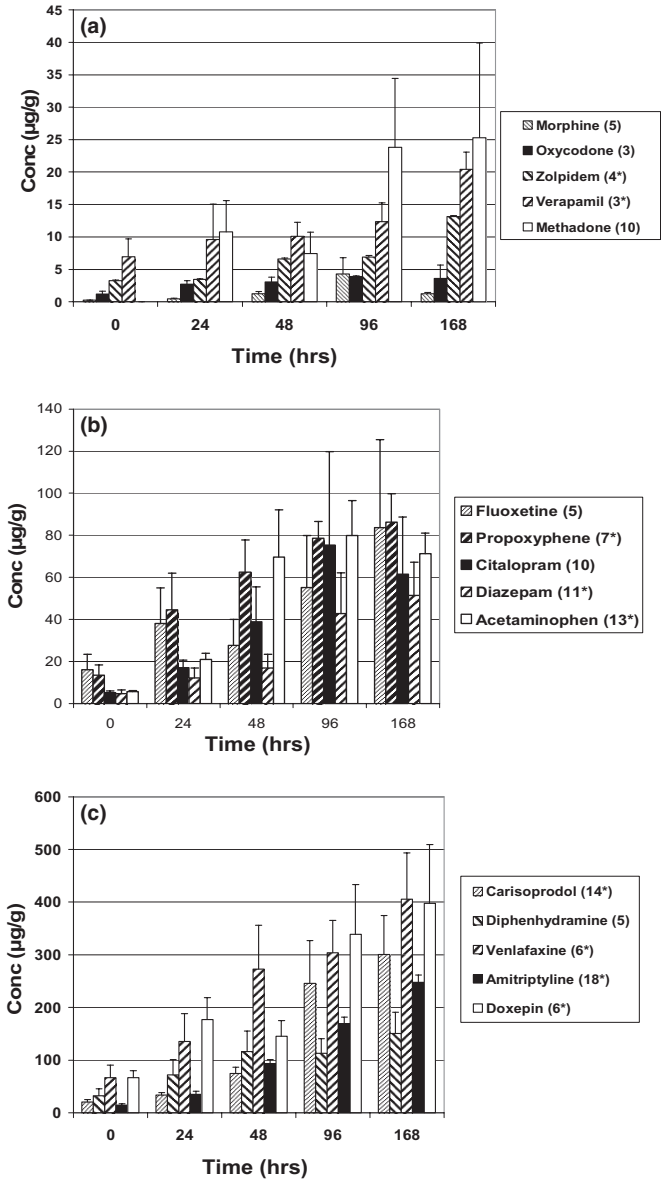

FIG. 4—*(a) Average temporal increase in morphine, oxycodone, zolpidem, verapamil, and methadone in decomposing liver. Error bars represent SEM, and statistical significance is indicated by *. (b) Average temporal increase in fluoxetine, propoxyphene, citalopram, diazepam, and acetaminophen in decomposing liver. Error bars represent SEM, and statistical significance is indicated by *. (c) Average temporal increase in carisoprodol, diphenhydramine, venlafaxine, amitriptyline, and doxepin in decomposing liver. Error bars represent SEM, and statistical significance is indicated by *.*

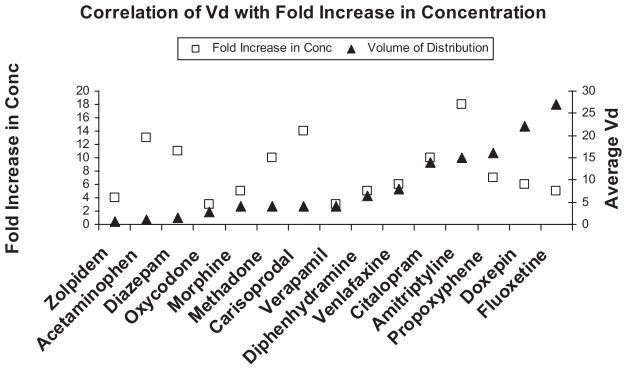

FIG. 5—*Correlation of fold increase in drug concentration in liver and average Vd (human).*

TABLE 3—*Average concentration of drugs (microgram per gram) in decomposing pig liver over time.*

	Time					Relative Increase	
Drugs	4 h	24 h	48 h	96 h	168 h	4–96 or 168 h	*p* Value*
Morphine	0.23	0.44	1.2	4.2		18	0.119
Amitriptyline	14	35	92	169	248	17	0.014
Citalopram	5.0	17	38	75	49	9.8	0.304
Diazepam	4.7	12	16	42	51	10	0.010
Methadone	2.5	10	7.8	23	25	10	0.081
Fluoxetine	16	38	27	55	83	5.2	0.060
Doxepin	66	177	146	339	398	6.0	0.011
Acetaminophen	5.0	20	66	74	63	12	0.002
Oxycodone	1.1	2.7	3.0	3.9	3.6	3.3	0.270
Diphenhydramine	33	72	116	113	151	4.6	0.129
Venlafaxine	67	135	273	304	405	6.0	0.012
Carisoprodol	21	38	139	216	300	14	0.001
Verapamil	6.9	9.6	10	12	20	3.0	0.145
Zolpidem	3.2	3.4	6.6	6.9	13	4.0	<0.0001
Propoxyphene	13	44	62	78	86	6.6	0.005

*Significance of the *F* statistic on the trend by one-way analysis of variance (ANOVA).

2 years after the study were amitriptyline and diazepam as well as pentobarbital which had been used as a euthanizing agent. Citalopram and morphine were not detected, which may reflect metabolism by microorganisms or enhanced water solubility in soil resulting in being washed away. Other sites of decomposition which may contain other drugs have not yet been investigated.)

An example of rapid loss of tissue weight for liver is shown in Fig. 2. A close correlation was observed between both necropsied and non-necropsied animals indicating that surgical removal of the livers and repetitive replacement in the carcass did not have a large effect on the organ mass over time.

The change in drug concentrations in decomposing muscle over time is shown in Fig. 3*a–c*. The values in parentheses, within the figure legends, represent the fold increase in concentration from time 0–96 h (or 168 h for Fig. 3*b*). Although all concentrations were higher for all drugs examined as decomposition progressed, the only increase in drug concentration found to be statistically significant was that of amitriptyline. These results are consistent with previous observations in rat thigh muscle (18).

Figure 4*a–c* depicts the effect of decomposition on drug concentration in liver. Compared to muscle, the concentration of drugs in liver tissue was substantially increased during a week of decomposition. All drug concentrations were increased but only 10 of the 16 drugs were increased to a statistically significant level (shown by *). The average concentration for each collection time and the level of statistical significance are shown in Table 3.

The difference observed between liver and muscle drug concentrations may be a result of a greater loss in tissue mass (most likely as fluids) for liver (see Fig. 1) than for muscle. Because drug concentration is expressed as microgram drug/gram tissue, a decrease in liver mass would result in an increase in drug concentration. However, attempts to normalize the data by calculating the total amount of drug in the entire liver still resulted in an increase in drug amount over time. Similarly, evaporation of all water from

TABLE 4—*Ascending order of average fold increase in drugs in muscle and liver.*

Drug		Drug	
Muscle	Fold Increase	Liver	Fold Increase
Carisoprodol	1.1	Verapamil	3
Diphenhydramine	1.3	Diphenhydramine	5
Fluoxetine	1.3	Fluoxetine	5
Venlafaxine	1.3	Venlafaxine	6
Diazepam	1.6	Doxepin	6
Verapamil	1.6	Propoxyphene	7
Methadone	1.6	Methadone	10
Doxepin	2	Citalopram	10
Propoxyphene	2	Diazepam	11
Citalopram	3.5	Carisoprodol	14
Amitriptyline	5.3	Amitriptyline	18

temporally collected liver specimens, by cold-trap centrifugation, and expression of concentration as microgram drug/gram dry liver also showed an increase in drug concentration with increasing decomposition time. However, the increase was not statistically significant.

The anatomic location of liver, compared to muscle, may explain part of the difference in drug concentration for the two decomposing tissues. Liver was in contact with the stomach and GI tract, and high concentration of drugs leaking from these decomposing organs may have added to the concentration found in liver. Elevated postmortem drug concentration in kidney of rats has been attributed to GI leakage (18).

The possibility that the difference in the amount of increase in drug concentration for different drugs might be a reflection of lipophilicity was examined by plotting the average volume of distribution of specific drugs (humans, [11]) against the relative increase in drug concentration. The total lack of correlation is shown in Fig. 5.

Whatever the mechanism, it is clear that the concentration of drugs analyzed increased with the state of decomposition. The increase in concentration was not the same for all drugs, and drug concentration in liver increased to a much greater extent than that in muscle. The variability in fold increase for drugs may reflect the relative stability of drugs in decomposing tissue. Along with an increase in concentration, there may be a concomitant, and variable, decrease in the amount of drugs because of biodegradation. This possibility is supported by the close correlation of the order of increase in drugs for both muscle and liver (see Table 4); exceptions to this correlation were carisoprodol and verapamil.

Conclusion

In conclusion, drug concentrations can dramatically increase in decomposing tissues; not knowing this may cause coroners or medical examiners to issue the mistaken diagnosis of drug overdose, either accidental or suicidal. The results of this study clearly show that at autopsy, high levels of drugs in decomposed bodies, considered in isolation, can neither support nor negate an overdose as the cause of death. However, one must consider the case information in its entirety. After having performed so, a high level of drugs in decomposed tissue may support a diagnosis of overdose.

Acknowledgments

The authors wish to express their appreciation to Sandy Beech, Franklin County Public Works for his assistance with cage construction and to Maureen Fagan and to Jeremy Pell, OSU Finlay Farm, for their support and assistance with animal handling and site maintenance. Also our thanks go to Nancy E. Tatarek, Ph.D., Department of Sociology and Anthropology, Ohio University, Athens, OH, for her help with data collection and to Calvin McGuire for his expert technical assistance with instrumentation maintenance.

This study was performed in accordance with the National Research Council's ''Guide for the Care and Use of Laboratory Animals'' and was approved by the ILACUC, The Ohio State University, Columbus, OH.

References

1. Paterson S. Drugs and decomposition. Med Sci Law 1993;33(2):103–9.
2. Drummer OH, Gerostamoulos J. Postmortem drug analysis: analytical and toxicological aspects. Ther Drug Monit 2002;24(2):199–209.
3. Pélissier-Alicot AL, Gaulier JM, Champsaur P, Marquet P. Mechanisms underlying postmortem redistribution of drugs: a review. J Anal Toxicol 2003;27(8):533–44.
4. Drummer OH. Postmortem toxicology of drugs of abuse. Forensic Sci Int 2004;142(2–3):109–13.
5. Flanagan RJ, Connally G. Interpretation of analytical toxicology results in life and at postmortem. Toxicol Rev 2005;24(1):51–62.
6. Drummer OH. Post-mortem toxicology. Forensic Sci Int 2007;165(2–3):199–203.
7. McIntyre LM, King CV, Boratto M, Drummer OH. Post-mortem drug analyses in bone and bone marrow. Ther Drug Monit 2000;22(1):79–83.
8. Flanagan RJ, Amin A, Seinen W. Effect of post-mortem changes on peripheral and central whole blood and tissue clozapine and norclozaine concentrations in the domestic pig (*Sus Scrofa*). Forensic Sci Int 2003;132(1):9–17.
9. Pond WG, Mersmann HJ, editors. Biology of the domestic pig. Ithica, NY: Comstock Pub, 2001.
10. Stevens HM. The stability of some drugs and poisons in putrefying human liver tissues. J Forensic Sci Soc 1984;24:577–89.
11. Baselt RC, editor. Disposition of toxic drugs and chemicals in man, 6th edn. Foster City, CA: Biomedical Pub, 2002.
12. Winek's Drug and Chemical Blood-Level Data 2001, http://medschool.slu.edu/abmdi/uploads/files/Winek%20tox%20data%202001.pdf.
13. Amendt J, Krettek R, Zehner R. Forensic entomology. Naturwissenschaften 2004;91:51–65.
14. UCT, Inc. Therapeutic and abused drugs in plasma/serum and urine for acid/neutral and basic drugs for GC or GC/MS confirmations using: 200 mg Clean Screen® extraction columns. In: ????? ?????, editors. 2009 UCT Solid Phase Extraction Applications Manual. Bristol, PA: UCT, Inc., 2008;42–3.
15. Dolinak D. Toxicology. In: Dolinak D, Matshes EW, Lew EO, editors. Forensic pathology principles and practice. London, U.K.: Elsevier Academic Press, 2005;489.
16. Lewis R, Johnson RD, Southern TL, Canfield DV. Distribution of butalbital in postmortem tissues and fluids from non-overdose cases. J Anal Toxicol 2003;27(3):145–8.
17. Garriott JC. Skeletal muscle as an alternative specimen for alcohol and drug analysis. J Forensic Sci 1991;36(1):60–9.
18. Shiota H, Nakashima M, Terazono H, Sasaki H, Nishida K, Nakamura J, et al. Postmortem changes in tissue concentrations of triazolam and diazepam in rats. Leg Med (Tokyo) 2004;6(4):224–32.

Additional information and reprint requests:
John F. Wyman, Ph.D.
Cuyahoga County Regional Forensic Science Laboratory
11001 Cedar Ave
Cleveland, OH 44106
E-mail: jfwyman@cuyahogacounty.us