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Original Article

Fate of Barbiturates and Non-steroidal Anti-inflammatory Drugs During Carcass Composting

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Abstract

With disease issues, the decline of the rendering industry, a ban on use of downer cows for food, and rules to halt horse slaughter, environmentally safe and sound practices for disposal of horses and other livestock mortalities are limited. Improper disposal of carcasses containing veterinary drugs has resulted in the death of domestic animals and wildlife. Composting of carcasses has been performed successfully to reduce pathogens, nutrient release, and biosecurity risks. However, there is concern that drugs used in the livestock industry, as feed additives and veterinary therapies do not degrade readily and will persist in compost or leachate, threatening environmental exposure to wildlife, domestic animals and humans. Two classes of drugs commonly used in the livestock and horse industries include barbiturates for euthanasia and non-steroidal anti-inflammatory drugs (NSAID) for relief of pain and inflammation. Sodium pentobarbital (a barbiturate) and phenylbutazone (an NSAID) concentrations in liver, compost, effluent and leachate were analyzed in two separate horse carcass compost piles in two separate years. Horse liver samples were also buried in 3 feet of loose soil in the first year and drug concentrations were assessed over time. In year one, phenylbutazone concentrations in the liver of the horse were undetectable (< 10 ppb) by 20 days of composting or burial in loose soil and were undetectable in effluent from the pile at the time of first sampling on day 6. Pentobarbital concentrations were undetectable (< 10 ppb) in liver samples retrieved from both the compost pile and loose soil by day 83. Rate of decay was faster in the soil, exponentially decreasing by 18% per day, with a half-life of 3 days, than in the compost pile where there was a 2% decrease per day and a half-life of 31 days, but occurred at the same rate of 1% and a half-life between 55 and 67 mesophilic degree days when calculated on the number of mesophilic degree days to which it was exposed. This suggests that breakdown of pentobarbital is not initiated by the heat of composting, but by the biological degradation that occurs in both soil and compost at mesophilic temperatures. Pentobarbital in the effluent decreased by 20% per day with a half-life of 3.1 days but was still detectable (0.1 ppm) at 223 days of composting. In year 2, phenylbutazone was not detected in any of the samples analyzed (compost and leachate) other than blood taken from the jugular vein of the horse immediately after euthanasia. Pentobarbital concentratoins in the compost were still detectable after 224 days of composting, but had decreased from 79.2 (initial) to 5.8 ppm. Pentobarbital in leachate was 2.2 ppm at day 56 of composting, after which no additional fluids leached into the leachate collection containers. Rate of decay in the leachate was 35.2% per day with a half-life of 1.6 days. When managed properly, composting will deter domestic and wild animals from scavenging on treated carcasses while they contain the highest drug concentrations providing an effective means of disposal of euthanized and/or NSAID treated livestock. The resulting compost contains either no or very low concentrations of both NSAIDs and barbiturates.

Keywords: *Mortality Composting, Carcass, Euthanasia, Barbiturates, NSAIDs, Animal Waste Disinfection*

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INTRODUCTION

Composting is a self-heating, aerobic process that accelerates the degradation of organic material by the successive action of a diverse group of microorganisms, including mesophiles, thermophiles, actinomycetes, and fungi. Because of the activity of microorganisms and the thermophilic temperatures reached, composting results in the destruction of pathogens in manure and carcasses (Bonhotal et al. 2006; Schwarz et al. 2010). However, pathogens are not the only concern in manure and mortality composting. Many pharmaceuticals used in livestock operations are not rapidly or completely degraded in the animal's body and can be present in the manure and animal tissue. Thus, their fate in composting can be a concern, depending on the ultimate use of the compost product. The fate of antibiotics, steroids and other hormones in manure composting has been studied (Arikan et al. 2007; Dolliver et al. 2008; Hakk et al. 2005). Little work, however, has been done on the fate of other veterinary drugs used in livestock and horse operations. Two such classifications of drugs are non-steroidal anti-inflammatory drugs (NSAID) and barbiturates. NSAIDs are used in the livestock industry as an analgesic, anti-inflammatory and antipyretic. One NSAID in particular, diclofenac, has caused the deaths of thousands of oriental white-backed vultures, causing near extinction, in Pakistan and India, where livestock that die are typically left for scavengers to remove, (Oaks et al. 2004; Cuthbert et al. 2011). Diclofenac is banned in the US, but it is unclear if other NSAIDs could have similar effects. There has been no research on the fate of NSAIDs in mortality composting. Properly built compost piles will deter scavengers from feeding on carcasses, but knowing the fate of NSAIDs in the compost pile is needed to assure appropriate use of the end product.

The American Veterinary Medical Association (AVMA 2001) reports the use of injectable euthanasia agents as the most rapid and reliable method of performing euthanasia, naming sodium pentobarbital as the best choice. However, they caution that "in euthanasia of animals intended for human or animal food, chemical agents that result in tissue residues cannot be used. Carcasses of animals euthanatized by barbituric acid derivatives or other chemical agents may contain potentially harmful residues." Barbiturate overdose of wildlife and domestic animals from feeding on carcasses that were improperly disposed has been reported by Anderson et al. (1979); Fucci et al. (1986); O'Rourke (2002) and Bischoff et al. (2011). In each of these cases, the carcass was either left uncovered or incomplete clean-up occurred. In addition, feeding of meat from horses euthanized with pentobarbital (both cooked and uncooked) has resulted in barbiturate poisoning of dogs (Polley and Weaver 1977; Reid 1978). In each of these cases, pentobarbital concentrations in the ingested tissue were approximately 30 ppm, an anesthetic dose. Rendering is not an acceptable form of disposal, as the rendering process does not eliminate pentobarbital from the tissue (O'Connor et al. 1985). Due to these and other incidents of similar nature, the US Food and Drug Administration (FDA 2003) added the following environmental warning to animal euthanasia products in 2003: "Environmental Hazard: This product is toxic to wildlife. Birds and mammals feeding on treated animals may be killed. Euthanized animals must be properly disposed of by deep burial, incineration, or other method in compliance with state and local laws, to prevent consumption of carcass material by scavenging wildlife."

There have been only a few studies to date on the fate of pentobarbital in mortality composting. Composite compost samples from 8 piles containing a euthanized equine carcass had pentobarbital residues remaining at 90 and 180 days and ranging from 0.008 to 3.16 ppm (Cottle et al. 2010). Work done by Middle Tennessee State University in Murfreesboro, TN, on aerobic decomposition of large-scale animal mortalities has shown that certain soil microorganisms that can be found in composting material have the ability to degrade barbiturates (M. Farone personal communication July 28, 2011). The purpose of this 2 year study was to determine the fate of phenylbutazone, an NSAID, and sodium pentobarbital, a barbiturate, during mortality composting to determine if the end product would not be detrimental to humans, other animals, or the environment during the compost process or when compost is used in agriculture.

MATERIAL AND METHODS

Year 1

Compost pile and temperature recording

On 9/22/09 a 6 x 6 m piece of 10 mil plastic sheeting was laid on bare soil with a 2% slope, at the edge of Cornell University's compost site in Ithaca, NY. Water was poured on the plastic to check the direction of flow. A hole was dug at the low end of the pad, under the plastic, large enough to fit a 76 l galvanized garbage can. A stainless steel container was placed in the garbage can to collect effluent. A hole was cut in the plastic over the container for collection. A 0.6 m high base (3.7 x 3.7 m) of coarse carbon material (mix of hard and soft woodchips) was laid on the plastic. A 27 year old Appaloosa mare, weighing approximately 455 kg that had been dosed with 1 gram phenylbutazone at midnight on 9/22/09 and again at 8:00 am was led onto the base and euthanized for severe lameness by a *licensed* veterinarian with 120 ml Fatal Plus® solution (active ingredient 390 mg/ml Pentobarbital Sodium). After the horse had been euthanized and the veterinarian ensured there were no signs of life, the carcass was maneuvered onto the wood chips with the head on the upward slope of the pad. The liver was removed from the horse and cut into 48 pieces, each weighing approximately 100 grams, and placed in nylon mesh bags which were then placed in whiffle balls. A 2 m length of nylon twine was attached to each ball. Twenty-three balls were inserted in the horse's gut cavity and 22 balls were placed in a 1 m hole in the ground (burial hole) which was dug approximately 1.5 m from the pad. Pieces of the intestine and some blood were also placed in the hole to help mimic the presence of a carcass. The remaining 3 nylon mesh bags with liver were packaged for delivery to Cornell University's Animal Health Diagnostic Center (AHDC) to determine initial NSAID and barbiturates concentrations. Two Hobo U12 data loggers with 4 temperature probes each were set up to record hourly temperatures. Five of the probes were placed in the compost pile: under the horse's chest, in the horse's hind gut, in the horse's chest cavity, under the horse's spine and under the horse's right hind quarter. Two of the probes were placed in the burial hole and one probe was left out to record ambient temperature. The hole was covered with loose soil. The horse was covered with woodchips so that the pile was approximately 1.8 m high. The plastic liner was tightened by rolling it over and under wooden fence posts.

Temperature and Rainfall

Hourly temperature data was downloaded from the data loggers through 4/29/10 (day 219). Average daily temperature was calculated and plotted along with rainfall amounts obtained from a local weather station. Thermophilic degree days, the number of days in which the temperature in the compost pile was between 40 and 65^{o}C, were calculated from hourly temperature readings in the following manner:

- An average of the hourly temperatures from the 5 probes in the compost pile was calculated.
- If the temperature was between 40 and 65^{o}C in that hour, the difference between actual and 40 was calculated as an hourly heat accumulation.
- These were then summed up over the study period and divided by 24 to give the number of thermophilic degree days in the compost pile.

Mesophilic degree days, the number of days in which the temperature in the compost pile and in the burial hole was between 4.4 and 40^{o}C was calculated in the same manner but instead by summing the hours in which the temperature was less than 40 but greater than 4.4^{o}C.

Sampling

Liver and Compost: Three whiffle balls with liver samples were pulled from both the compost pile and the burial hole at days 6, 20, 41, 83 and 181 and delivered to AHDC for quantitative analysis of phenylbutazone and sodium pentobarbital. In addition, at day 181, 2 samples were taken from the area directly around where the balls had been placed, resulting in a sample of paunch manure mixed with woodchips. At day 219, the pile was mixed and 2 samples of compost were taken.

Effluent: Effluent samples (runoff and leachate) were taken in duplicate from the container pan after rainfall events at days 6, 15, 34, 41, 59 and 223 and delivered to AHDC for quantitative analysis of phenylbutazone and sodium pentobarbital. Since the horse had been dissected to collect liver samples, and over 50 mm of rain fell in the first 8 days of composting, the pan used to collect effluent overflowed several times. Therefore, these samples contained more than just the leachate and runoff, but also included blood, woodchips, soil, etc.

Laboratory Analysis

Liver, Compost Extraction: Samples were mixed using a homogenizer prior to sub-sampling. A 5.0 g portion of liver or compost was weighed and placed into a 25 x 150 mm screw cap test tube fitted with Teflon lined caps. A standard curve for pentobarbital and phenylbutazone (10.0 ppb -10.0 ppm) was obtained by spiking 5 g of control samples with pentobarbital and phenylbutazone at (0.0, 10.0, 25.0, 50.0, 100.0 ng/g, and 1.0 and 10 µg/g if needed) using the working standard. Additional sample spikes were analyzed depending on range of pentobarbital and phenylbutazone concentration in samples analyzed. All samples and sample spikes were extracted as follows:

- Sample pH was adjusted using 20.0 ml of basic deionized water (pH 10) added to each sample and control spike.
- Samples were mixed on a Rotorack for 10 minutes.
- The water extracts were filtered into clean, labeled 25 x 150 mm screw cap tubes.
- The water extracts were adjusted to pH 3.5 using drop wise additions of 0.6N HCl.
- Samples were extracted with 10.0 ml of Tri-solvent (Ethyl Ether/Hexane/Dichloromethane 1:1:1) by mixing 10 minutes on a Rotorack.
- Samples were centrifuged at 3000 x g.
- The top organic layer was transferred to a 13 x 100 mm screw cap test tube, placed in a 60^{o}C water bath and concentrated to dryness under a gentle flow of nitrogen.
- Samples were diluted with 0.5 ml ethylacetate and sonicated for 1 minute.
- A 1 µl volume of sample extract was injected using a splitless injection into the capillary GC/MS system

(Agilent 5973N) equipped with electronic pressure control.

- The injector temperature was 250°C. Peak retention time for pentobarbital and phenylbutzaone was 9.60 and 12.29 min, respectively.
- The area under the curve was calculated to determine the total pentobarbital or phenylbutazone concentration in the samples.

Effluent Extraction: 5.0 ml of effluent water sample was transferred to a labeled 25 x 150 mm screw cap test tube. The effluent was adjusted to pH 3.5 by drop wise additions of 0.6N HCl. A standard curve for pentobarbital and phenylbutazone (10.0-100.0 ppb) was obtained by spiking 5 ml of deionized water with pentobarbital and phenylbutazone at (0.0, 10.0, 25.0, 50.0 and 100.0 ng/g) using the working standard. Samples were extracted using 10.0 ml Trisolvent (Ethyl Ether/Hexane/Dichloromethane 1:1:1) by mixing on a Rotorack for five minutes. The top organic layer was transferred to a 13 x 100 mm screw cap test tube, placed in a 60°C water bath and concentrated to dryness under a gentle flow of nitrogen. The extracts were cleaned up using Florisil columns to remove fat. Samples were reconstituted with 0.5 ml of ethyl acetate, sonicated for 1 minute and analyzed by capillary GC/MS as above.

Statistical Analysis

Determination of differences between mean phenylbutzaone and mean pentobarbital concentrations at differing sample dates was conducted using standard least square means analysis with Tukey corrections at a significance level of $p = 0.05$. Subsequent determination of exponential decay of phenylbutazone and pentobarbital over time and by thermophilic and mesophilic degree days in the liver samples collected from both the compost pile and the burial hole was conducted using nonlinear regression with $\alpha = 0.05$. Linear regression on the log transformation of phenylbutazone and pentobarbital concentrations over time and by degree days was then performed to test for significance of the exponential decay. Exponential decay of both drugs was also performed on the values generated from the effluent samples. All statistical analysis was conducted using JMP 9.0.0 software (SAS Institute Inc., Cary, NC, 2010).

Year 2

Leachate Collection Troughs

In year 1, the collection of "leachate" included precipitation that diluted the leachate. In year 2, to target only the liquids that leached out of the horse and through the pile, two 3 m long troughs with a 1% slope were built out of 15 and 10 cm diameter PVC pipe attached to 5 x 15 cm untreated lumber. The troughs were placed on the pad from the centerline to the edge of the pile end-to-end with slopes going toward the outside of the pile. Leachate drained via gravity into 2-liter polyethylene bottles attached to the troughs. The exposed ends of the troughs were covered with 1 m length of aluminum flashing to keep rainwater out of the collection bottles.

Horse Compost Pile and Temperature Recording

On 8/10/10 the leachate collection troughs were laid on bare soil with a 2% slope at the edge of Cornell University's compost site in Ithaca, NY. A 0.6 m high base (3.7 x 3.7 m) of coarse carbon material (mix of hard and soft woodchips) was laid on top and around the troughs so as to envelop them in the material. A 22 year old horse weighing approximately 590 kg, that had been dosed with 1 gram phenylbutazone at midnight on 08/10/10 and again at 7:30 am, was led onto the base and euthanized by a licensed veterinarian with 300 mg xylazine as a sedative, then with 120 ml Fatal Plus® solution (active ingredient 390 mg/ml Pentobarbital Sodium). After the horse had been euthanized and the veterinarian ensured there were no signs of life, the carcass was maneuvered on the wood chips with the head on the upward slope of the pad. The veterinarian took 4 tubes of blood from a vein in the nose and a vein in the front leg of the horse in heparinized Vacutainer® tubes for initial concentrations of pentobarbital and phenylbutazone. Twenty-six whiffle balls that had been pre-filled with wood chips (the base material of the compost pile) were placed such that they would be under the horse and liquids coming from the horse would be absorbed by the chips inside the balls, as well as in the surrounding base material, while the excess would drain down the leachate collection troughs and be captured in the 2 liter bottles at the end of the troughs (Figure 1). One Hobo U12 data logger with 4 temperature probes was set up to record hourly temperatures. The probes were placed under the horse's neck and rump, on top of the horse's abdomen, and one was left out to record ambient temperature. The horse was covered with woodchips so that the pile was approximately 1.8 m high. Additional woodchips were added to the pile on August 13 and the pile was covered with a breathable polyester compost cover to collect only what was leaching from the animal.

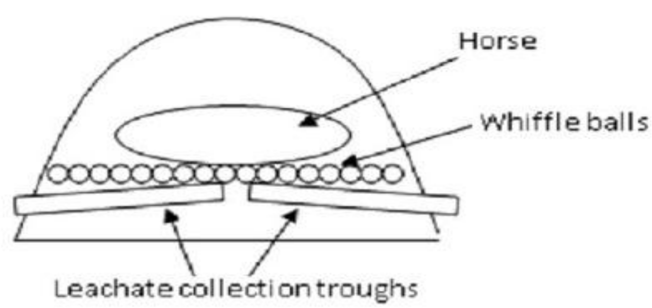

Figure 1 Cross-section of horse compost pile showing placement of leachate collection troughs and woodchip-filled whiffle balls

Cow Compost Pile and Temperature Recording

On 8/10/10 a 0.6 m high base (3.5 x 3.5 m) of coarse carbon material was laid near the horse compost pile. A 455 kg 3 year, 7 month old, 2^{nd} lactation Holstein cow was euthanized, due to a lung abscess, in the same manner as the horse (300 mg xylazine, followed by 120 ml Fatal Plus®). Four tubes of blood were withdrawn from her milk vein as described for the horse. One Hobo U12 data logger with 4 temperature probes was set up to record hourly temperatures. The probes were placed under the cow's udder and rear leg, on top of the cow's back, and one was left out to record ambient temperature. The cow was then covered with woodchips so that the pile was approximately 1.8 m high. Additional woodchips were added to the pile the following day before the pile was covered with a compost cover.

Temperature and Rainfall

Hourly temperature data was downloaded from the data loggers through 3/22/11 (day 224). Average daily temperature in both the horse and cow compost piles were calculated and plotted along with rainfall amounts from a local weather station. Thermophilic and mesophilic degree days were calculated from hourly temperature readings as previously described.

Sampling

Woodchips/Compost: Two whiffle balls with woodchips were pulled from the horse compost pile at days 7, 10, 14, 17, 28, 56, 84, 126 and 161 and delivered to AHDC for quantitative analysis of phenylbutazone and sodium pentobarbital. In addition, on day 224, the horse compost pile was mixed with a backhoe and 2 compost samples were taken. The cow pile was not mixed at this time, but two samples of compost were taken close to where the data logger probes had been placed.

Leachate: The horse pile was monitored daily through day 20 for leachate in the collection containers. In that time period, if there was leachate in the bottles, the amount was measured and a subsample was brought to AHDC for analysis of phenylbutazone and pentobarbital. After day 20, the pile was monitored weekly for leachate. As a result, samples were taken on days 3, 6, 7, 10, 14, 17, 30 and 56. After day 56, no more leachate was found in the collection containers. On day 3, the laboratory analyzed only for pentobarbital.

Soil: On day 56, a hole was dug through the pile containing the cow to the underlying soil where 2 soil samples were taken. On day 224, while the horse pile was mixed, 2 soil samples were taken from a spot as close to the center of the horse pile as possible. In addition, the cow pile was pushed back with a backhoe so that 2 soil samples could be taken from underneath that pile. All soil samples were analyzed for phenylbutazone and pentobarbital.

Laboratory Analysis

Samples, including soil, were analyzed as described in year 1.

Statistical Analysis

Determination of differences between mean phenylbutzaone and mean pentobarbital concentrations at differing sample dates was conducted using standard least square means analysis with Tukey corrections at a significance level of $p = 0.05$. Exponential decay of the level of sodium pentobarbital in the leachate samples was conducted using nonlinear regression with $\alpha = 0.05$. All statistical analysis was conducted using JMP 9.0.0 software (SAS Institute Inc., Cary, NC, 2010).

RESULTS AND DISCUSSION

Climate, Temperature and Degree Days
Year 1

Air Temperature and Precipitation: Mean daily air temperatures were 0 to 20^{o}C during the composting period except for 2 cold periods during the month of December and mid-January to mid-February when mean air temperatures were 0 to -15^{o}C (Figure 2). A total precipitation (rain and snow) of 508 mm was recorded through day 219 of composting. Precipitation was fairly evenly distributed over that period, however, over 50 mm of rain fell in the first 8 days after building the pile, which, combined with the blood released while dissecting the horse, made effluent collection difficult.

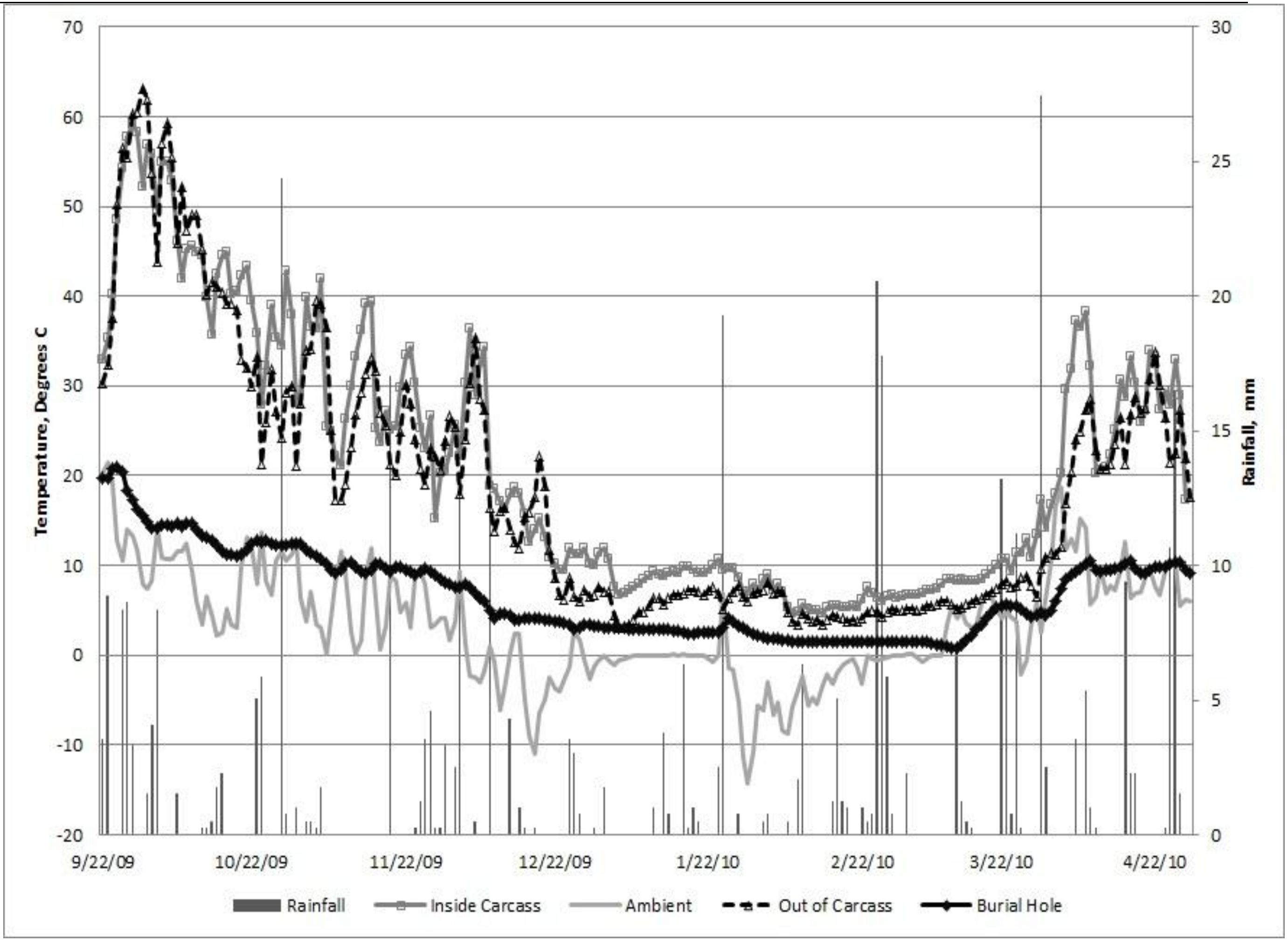

Figure 2 Mean daily air temperature, precipitation and temperature in and out of the carcass in the compost pile and in the "burial" hole during the composting period, September 2009 – April 2010

Horse Pile: Under optimal conditions, composting occurs in 3 phases according to temperature during which different communities of microorganisms predominate (Trautmann and Olynciw 1996). The first phase, which typically lasts for a couple of days, is the mesophilic, or moderate-temperature phase (10-40^{o}C). During this phase mesophilic microorganisms rapidly break down the soluble, readily degradable compounds producing heat. This is followed by a thermophilic or high-temperature phase (40-65^{o}C) that can last from weeks to several months depending on the size of the system and the composition of the ingredients. This thermophilic stage is necessary for pathogen kill. The final phase is a 60-180 day mesophilic curing or maturation phase. When a pile is turned, effectively aerating it, temperatures tend to increase. It can be seen in Figure 2 that these three stages were met both in and outside of the carcass. The first mesophilic phase lasted approximately 3 days and was followed by a 27-day thermophilic phase. During the rest of the composting period, temperatures within the pile ranged between 4 and 38^{o}C, even when the ambient temperature was well below zero. This pattern indicates that composting occurred as expected, temperatures required for effective pathogen kill were met and all soft tissue was degraded. As previously described in the materials and methods section under temperature and rainfall, thermophilic and mesophilic degree days for the pile were calculated (Figure 3). There were 214 thermophilic degree days in the compost pile over the 219 calendar day composting period and 4,447 mesophilic degree days.

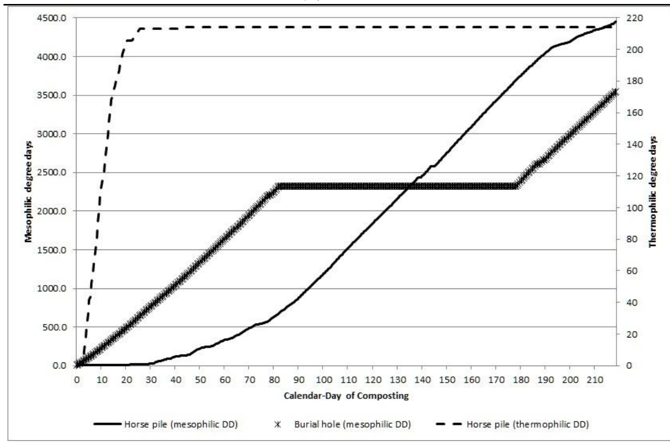

Figure 3 Number of mesophilic (primary y-axis) and thermophilic (secondary y-axis) degree days in the horse carcass pile and burial hole over 219 calendar-day composting period

"Burial" Hole: Temperatures in the 3 foot hole (Figure 2) were fairly constant starting at about 20°C in the warmer days of autumn, dropping to 1°C during the winter and coming back up to 10°C as spring approached. As thermophilic temperatures were not reached, there were 0 thermophilic degree days and 3,547 mesophilic degree days over 219 days in loose soil (Figure 3).

Year 2

Air Temperature and Precipitation: Mean daily air temperatures were 0 to 25°C during the first 3 months of composting. For the rest of the composting period mean air temperatures were between 10 and -22°C (Figure 4). A total precipitation (rain and snow) of 2342 mm was recorded through day 223 of composting, with almost 75% of it falling as snow from January through March, 2011.

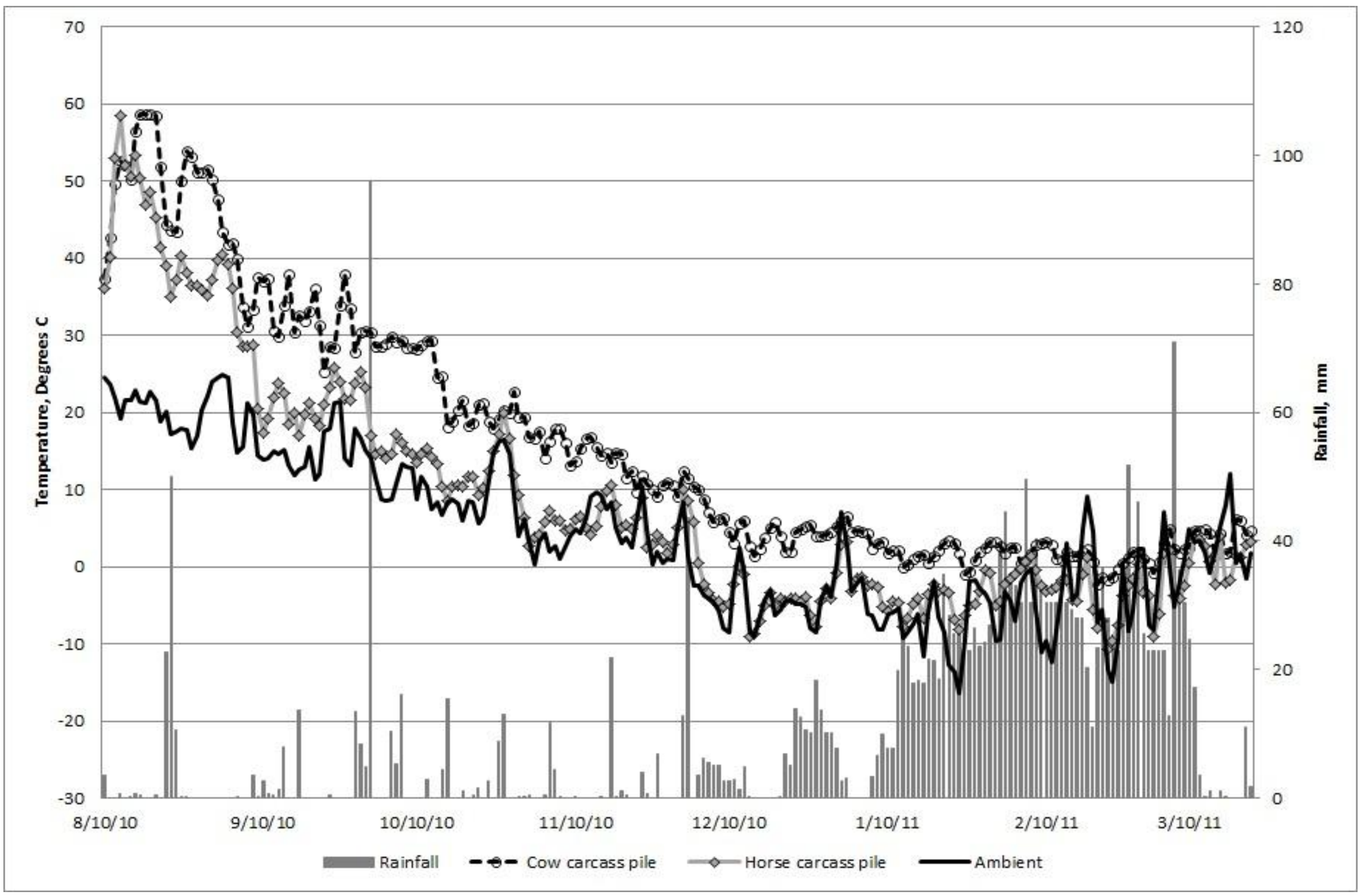

Figure 4 Mean daily air temperature, precipitation and temperature in cow and horse carcass compost piles during the composting period, August 2010 - March 2011

Horse and Cow Piles: It can be seen in Figure 4 that the three stages of composting were met for both the cow and the horse carcass piles. The first mesophilic phase lasted 1 day in both piles and was followed by a 24 day thermophilic phase in the cow pile and a 10 day thermophilic phase in the horse pile. The horse pile had a shorter thermophilic phase than the cow pile because more air circulated through the pile due to the preferenctial air channels created by the leachate collection troughs. Therefore, the pile received more air and dried out more than if leachate had not been collected. During the rest of the composting period, temperatures within the piles ranged between -10 and 39^{o}C with the lowest temperatures occurring under unusually low ambient temperature conditions. As previously described, thermophilic and mesophilic degree days for the piles were calculated. There were 258 and 94 thermophilic degree days in the cow and horse compost piles, respectively, and 5,214 and 2,183 mesophilic degree days over the 223 day composting period.

Phenylbutzaone Concentration

Year 1

Liver and Compost: Average phenylbutazone concentrations in compost and the liver samples retrieved from the compost pile and the burial pit are reported in Table 1. The liver, immediately after euthanasia, averaged 4.2 ppm phenylbutazone. Phenylbutazone concentrations in the liver samples decreased to below the detection limit of 0.001 ppm by day 20 in the burial hole and by day 41 in the compost pile. At day 6, phenylbutazone concentrations in the liver in both the burial hole and the compost pile were significantly lower (89 and 67%, respectively) than they were immediately following euthanasia. Exponential decay of phenylbutazone in the liver samples buried in loose soil occurred at a rate of 29.8% per day with a half-life of 2.3 days ($p < .0001$, $r^2 = 0.9955$) and at a rate of 16.0% per day with a half-life of 4 days for liver samples from the compost pile ($p < .0001$, $r^2 = 0.9856$).

Phenylbutazone is rapidly metabolized by microsomes (subcellular fractions of hepatocytes that contain drug-metabolizing enzymes) in the liver (Plumb 2008); the elimination half-life is 3 – 10 hours in horses (Boothe 2001). Enzymes in the liver will still function for up to 3 days after death (W. Schwark personal communication 6/15/11), thus drugs that are rapidly metabolized in the liver should continue to degrade after death. Phenylbutazone concentrations were non-detectable (< 0.001 ppm) in the compost samples taken at days 181 and 219.

Effluent: Phenylbutazone concentrations were non-detectable in all of the effluent samples taken. This can be explained by the fact that in horses, 96-99% of phenylbutazone is bound to plasma proteins, leaving very little to be metabolized or excreted in the effluent (Boothe 2001).

Table 1 Average phenylbutazone concentrations (ppm) in compost and liver samples retrieved from the compost pile and burial hole over time

Date	Day	Compost	Pile Liver	Burial Hole Liver
09/22/09	0		4.2^{a}	4.2^{a}
09/28/09	6		1.5^{b}	0.5^{c}
10/12/09	20		0.03^{c}	$< 0.001^{c}$
11/02/09	41		$< 0.001^{c}$	$< 0.001^{c}$
12/14/09	83		$< 0.001^{c}$	$< 0.001^{c}$
03/22/10	181	< 0.001	$< 0.001^{c}$	$< 0.001^{c}$
04/29/10	219	< 0.001		

Values with different superscripts are significantly different ($p < 0.0001$, $r^2 = 0.9868$)

Year 2

Blood, Woodchips/Compost and Soil: The blood taken from the horse contained 0.009 ppm phenylbutazone. After that initial sampling, phenylbutazone was below the detection limit of < 0.001 for all samples taken. In contrast to year 1, where phenylbutazone was detected in samples from the compost pile and burial hole at 20 and 6 days, respectively, no phenylbutazone was detected in the woodchips. This may be because the horse was still intact and no tissue or fluids would have mixed with or leached onto the woodchips placed under the horse when phenylbutazone was still present in the liver or bloodstream. No phenylbutazone was detected in any of the soil samples taken.

Leachate: As previously described, leachate was collected via polyethylene bottles attached to leachate collection troughs placed under the horse. In addition, the pile was covered with a compost cover so that all of the liquid collected in the bottles should reflect only what was leaching from the horse and the pile. As with the effluent from year 1, phenylbutazone was not detected in any of the leachate samples taken.

Pentobarbital Concentration

Year 1

Liver and Compost: Average pentobarbital concentrations in compost and liver samples retrieved from both the compost pile and the burial pit are reported in Table 2. The liver, immediately after euthanasia, averaged 92.6 ppm pentobarbital. Pentobarbital concentrations in the liver samples decreased to below the detection limit of 0.001 ppm by day 83 in both the burial hole and the compost pile. At day 6, pentobarbital concentrations in liver samples from the burial hole were significantly lower (73%) than they were immediately following euthanasia, but it took until day 41 for a similar difference to occur in liver samples pulled from the compost pile. Pentobarbital concentrations in the compost samples taken at days 181 and 219 were < 0.001 and 0.17 ppm, respectively.

Table 2 Average pentobarbital concentrations (ppm) in compost and liver samples retrieved from the compost pile and burial hole over time

Date	Day	Compost	Pile Liver	Burial Hole Liver
09/22/09	0		92.6^{ab}	92.6^{ab}
09/28/09	6		74.5^{b}	25.2^{c}
10/12/09	20		111.4^{a}	11.7^{c}
11/02/09	41		20.2^{c}	8.6^{c}
12/14/09	83		$< 0.001^{c}$	$< 0.001^{c}$
03/22/10	181	< 0.001	$< 0.001^{c}$	$< 0.001^{c}$
04/29/10	219	0.17		

Values with different superscripts are significantly different ($p < 0.0001$, $r^2 = 0.9868$)

As shown by the length of time before a significant difference in liver pentobarbital concentration occurred in the compost pile, exponential decay of pentobarbital in the liver samples buried in loose soil was significantly faster (18.4% per day with a half-life of 3.4 days) than that of liver samples from the compost pile (2.2% per day with a half-life of 30.9 days). This can be explained by the fact that aerobic soil biodegradation is the major pathway of degradation of pharmaceutical drugs in the soil leading to partial or complete mineralization and/or biotransformation (Velagaleti 1997). Pharmaceutical drugs biotransform and degrade completely in soils more rapidly than in water because of the diversity of microorganisms present in soils (fungi, bacteria, and actinomycetes). These miccroganisms are also present in the compost pile, but different organisms are present during the thermophilic stage than in the mesophilic stage. In a study to isolate and identify microorganisms that assist in the breakdown of sodium pentobarbital used in euthanasia during aerobic decomposition, Farone (2011) isolated 2 different bacteria that show enhanced growth on barbital containing media, indicating pentobarbital degradation. One of these organisms is a "safe" organism that came directly from soil and could be found in compost material (M. Farone personal communication July 28, 2011). Being a soil microorganism, it functions at mesophilic temperatures, indicating that it would not be present in compost during the thermophilic phase. Therefore, exponential decay of pentobarbital in the liver samples was calculated on mesophilic degree days in the samples taken from the burial hole, where only mesophilic temperatures were achieved and both thermophilic and mesophilic degree days on the samples taken from the compost pile. Table 3 shows the number of mesophilic and thermophilic degree days at each sampling date for both the burial hole and compost pile.

Table 3 Total mesophilic and thermophilic degree days in the burial hole and compost pile at each sampling date

Date	Day	Burial Hole	Compost Pile	
		Mesophilic degree days	Mesophilic degree days	Thermophilic degree days
09/22/09	0	0	0	0
09/28/09	6	132	11	52
10/12/09	20	488	11	205
11/02/09	41	1072	122	213
12/14/09	83	2318	704	214
03/22/10	181	2424	3784	214
04/29/10	219	3547	4447	214

Concentration of pentobarbital in the liver samples in the burial hole decreased 1% per mesophilic degree day with a half-life of 67 mesophilic degree days (Figure 5). The pile was built on 9/22/09 at 13:00 hours. On 9/25/09 at 22:00 hours, 67 mesophilic degree days had been reached. That is 3 days and 9 hours or 3.4 days (Figure 3). This corresponds with the 3.4 days previously calculated based on days of study. This explains why it took longer (in terms of days of study) for pentobarbital concentration in liver samples pulled from the compost pile to decrease.

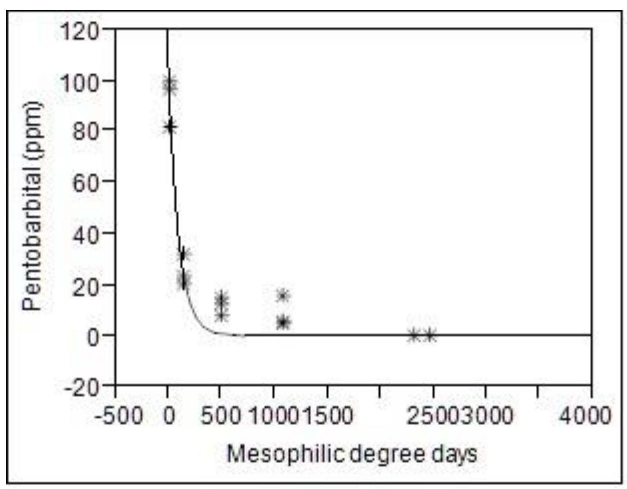

Figure 5 Exponential decay of pentobarbital in liver samples from the burial hole by mesophilic degree days ($p = 0.0009$, $r^2 = 0.6836$)

Figure 6 shows that there is no correlation between pentobarbital concentration and thermophilic degree days but there is a correlation between pentobarbital concentration and mesophilic degree days. Pentobarbital concentrations in liver samples pulled from the compost pile also decreased by 1% per mesophilic degree day with a half-life of 57 mesophilic degree days. However, it took 33 calendar days to reach 57 mesophilic degree days. The pile was built on 9/22/09 at 13:00 hours. On 10/25/09 at 21:00 hours, 57 mesophilic days were reached. (33.3 days, Figure 3). This corresponds with the 30.9 days previously calculated based on days of study.

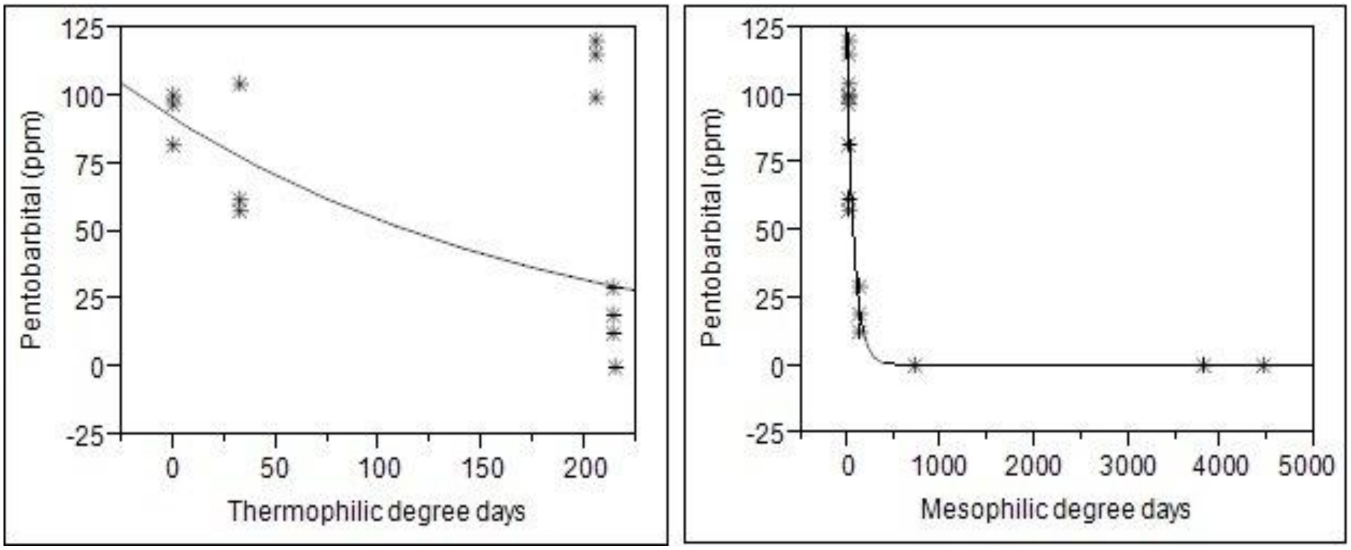

Figure 6 Exponential decay of pentobarbital in liver samples from the compost pile by thermophilic ($p = 0.0875$ not significant) and mesophilic degree days ($p < 0.0001$, $r^2 = 0.9206$)

Effluent: Pentobarbital concentration in the effluent on the days it was collected is shown in Table 4. The data begins on day 6 when the first effluent sample was collected. The concentration of pentobarbital in the leachate dropped approximately 7-fold between day 6 and day 15 then remained at the day 15 concentration throughout the remainder of the collection period. As there was no day 0 value, exponential decay could not be calculated, but there appears to be a quick and sustained decrease in pentobarbital concentration. However, since effluent was collected, rather than leachate, this data may be of little value. Barbiturates are hygroscopic and will decompose with exposure to air, heat, and light (Branson 2001), so any pentobarbital that leached with body fluids into the effluent would be expected to also decompose quickly.

Table 4 Average pentobarbital concentrations (ppm) in effluent over time

Date	Day	Pentobarbital Concentration
09/28/09	6	0.561[a]
10/07/09	15	0.086[b]
10/26/09	34	0.010[b]
11/02/09	41	0.092[b]
11/20/09	59	0.016[b]
05/03/10	223	0.100[b]

Values with different superscripts are significantly different ($p < 0.0001$, $r^2 = 0.9868$)

Year 2

Woodchips and Compost: Average pentobarbital concentrations from blood at day 0, woodchips at days 7 through 161 retrieved from the compost pile, and mixed compost at day 224 are reported in Table 5. The blood, immediately after euthanasia, contained an average of 110 ppm pentobarbital. Exponential decay is not appropriate here as there is no time 0 sample of woodchips with which to compare those values later in time. Pentobarbital concentratoins in the woodchips sampled were significantly lower than the concentration in the blood at all sampling dates and not significantly different from each other. Cottle et al. (2010) also found no difference in pentobarbital concentrations between sampling dates and concluded that pentobarbital is not broken down during the composting process, but they did not have a starting concentration with which to compare those values. Pentobarbital concentrations in the mixed compost samples taken at day 224 averaged 5.84 ppm, a 14-fold decrease from what was administered for euthanasia. According to Branson (2001) a lethal dose of pentobarbital in the dog is 85 mg/kg body weight orally and 28 - 30 mg/kg body weight would need to be consumed for an anesthetic response. Even at day 161, when average pentobarbital concentrations were 11.7 ppm (mg/kg compost), an average size dog of 20 kg would need to eat a total of 50 kg of the compost/woodchips (585 mg pentobarbital) in order to consume the anesthetic dose of 30 mg/kg body weight (600 mg).

Table 5 Average pentobarbital concentrations (ppm) in blood, woodchip samples retrieved from the compost pile, and compost over time

Date	Day	Blood	Woodchips	Compost
08/10/10	0	110[a]		
08/17/10	7		0.42[b]	
08/20/10	10		0.65[b]	
08/24/10	14		0.98[b]	
08/27/10	17		0.36[b]	
09/07/10	28		1.62[b]	
10/05/10	56		5.70[b]	
11/02/10	84		0.51[b]	
12/14/10	126		3.58[b]	
01/18/11	161		11.65[b]	
03/22/11	224			5.84[b]

Values with different superscripts are significantly different ($p < 0.0001$, $r^2 = 0.9885$)

Leachate: Table 6 shows the volume of leachate collected from the troughs beginning on day 3 and going through day 56, after which no more leachate was found in the bottles connected to the troughs. A total of 6.6 liters of leachate was collected over the course of the study. Assuming that the volume of body fluids in the horse is 60 – 70% of the horse's body weight, a 590 kg horse would have 354 – 413 l body fluids. The amount of fluids leaching from the pile represented approximately 1.7% of total fluids, with the rest of it being absorbed by the woodchips in the compost pile. Pentobarbital concentration in the leachate on the days it was collected is also shown in Table 6. Exponential decay of pentobarbital concentrations in leachate occurred at a rate of 35.2% per day, with a half-life of 1.6 days and had decreased 50-fold by day 56.

Table 6 Volume (ml) of leachate collected from the compost pile and average pentobarbital concentration (ppm) over time

Date	Day	Volume	Pentobarbital Concentration
08/10/10	0*	0	110.0
08/13/10	3	2700	13.7
08/17/10	7	600	41.2
08/20/10	10	1475	4.1
08/24/10	14	150	6.2
08/27/10	17	50	2.4
09/09/10	30	800	2.8
10/05/10	56	800	2.2

***Day 0 sample is pentobarbital concentration in the blood taken immediately after euthanasia**

Soil: At day 56, the soil under the pile in which the cow was composting averaged 0.2 ppm pentobarbital. By day 224, the amount had decreased 10-fold averaging 0.02 ppm in the soil under both piles. The fact that soil contained some pentobarbital early on indicates that the drug does reach the soil, most likely through fluids leaching from the carcass, but as shown by results from the liver samples buried in loose soil, once in the soil, the microorganisms residing there break down the pentobarbital.

CONCLUSIONS

Barbiturate poisoning in domestic and wild animals has occurred from ingestion of tissue from animals euthanized with pentobarbital. Many of the reported cases have occurred from direct feeding on improperly disposed livestock in which little or no degradation or biotransformation of pentobarbital has occurred. During the time period in which carcasses would be desirable to domestic and wild animals as a food source, composting creates sufficient heat to deter them from digging in to the pile. In addition, when covered properly, the smell of decomposition is minimized, also reducing attraction. The diverse community of microorganisms in the compost pile aids in the degradation and biotransformation of pentobarbital, especially after the thermophilic phase of composting is over. Properly implemented composting, as a means of disposal of euthanized or NSAID treated livestock, will deter domestic and wild animals from scavenging for carcasses when they contain the highest drug concentrations. The resulting compost contains either no or very low concentrations of either NSAIDs or barbiturates.

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