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Quantification of sodium pentobarbital residues from equine mortality compost piles[1]

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ABSTRACT: Sodium pentobarbital, a euthanasia drug, can persist in animal carcasses following euthanasia and can cause secondary toxicosis to animals that consume the remains. This experiment was conducted to observe the effects of composting on euthanized horse carcass degradation and sodium pentobarbital residues in compost material up to 367 d. Six separate compost bins were constructed on pastureland. Three bins served as the control while 3 served as the treatment. The carbonaceous material, or bulking agent, consisted of hardwood chips mixed with yard waste wetted to approximately 50% moisture content. Bulking agent was added to each bin at a depth of 0.46 m, creating the pad. A licensed veterinarian provided 6 horse carcasses for use in the experiment. These horses had required euthanasia for health reasons. All horses were weighed and then sedated with an intravenous injection of 8 mL of xylazine. After sedation the 3 horses in the treatment group were euthanized by intravenous injection of 60 mL of sodium pentobarbital. The 3 control group horses were anesthetized by intravenous injection of 15 mL of ketamine hydrochloride and then humanely euthanized by precise gunshot to the temporal lobe. Following euthanasia, each carcass was placed on the center of the pad and surrounded with 0.6 m of additional bulking agent. Serum and liver samples were obtained immediately following death. Compost samples were obtained on d 7, 14, 28, 56, 84, 129, 233, and 367 while soil samples were obtained on d -1 and 367. Each sample was analyzed for sodium pentobarbital concentration. Compost pile and ambient temperatures were also recorded. Composting successfully degraded soft tissue with only large bones remaining. Data illustrate that sodium pentobarbital was detectable up to 367 d in compost piles with no clear trend of concentration reduction. Drug residues were detected in soil samples indicating that sodium pentobarbital leached from the carcass and through the pad. These findings confirm the persistence of sodium pentobarbital from equine mortality compost piles and emphasize the importance of proper carcass management of animals euthanized with a barbiturate to reduce environmental impact and secondary toxicosis.

Key words: animal mortality, carcass disposal, composting, euthanasia, horse, sodium pentobarbital

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INTRODUCTION

For horses suffering from an incurable illness or injury, euthanasia is often the most humane option. The American Veterinary Medical Association (**AVMA**) approved methods for horse euthanasia include barbiturate overdose and captive bolt or gunshot to the temporal lobe (AVMA, 2013). Improper disposal of animal carcasses can present potential environmental, animal, and public health risks. Composting livestock carcasses is a biosecure and environmentally sound approach to carcass disposal when properly managed (Kalbasi et al., 2005; Wilkinson, 2007).

Recent interest has focused on the common euthanasia barbiturate, sodium pentobarbital, and its persistence in the animal carcass following euthanasia. Barbiturates accumulate within the carcass and can cause sedation or death of animals that may consume the remains (O'Rourke, 2002; Kaiser et al., 2010; AVMA, 2013). In 2003, the FDA added environmental

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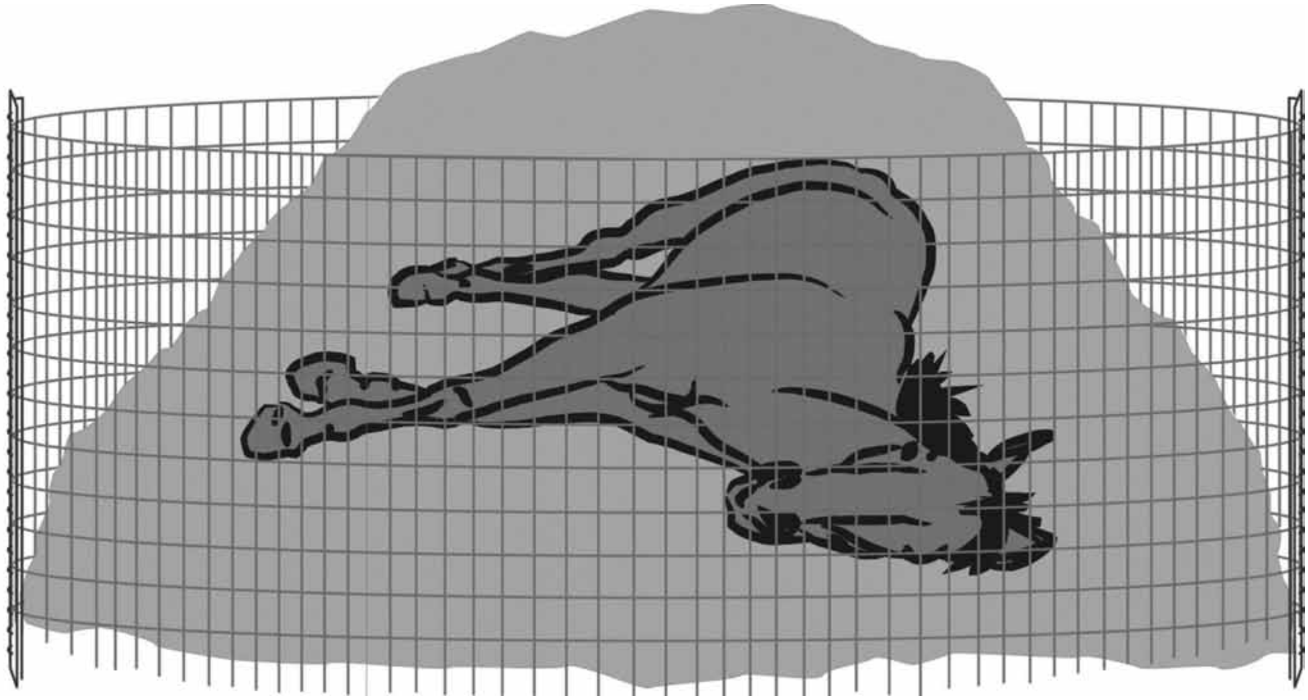

Figure 1. Illustration of compost bin constructed with metal horse panels and steel t-posts.

warning labels to euthanasia products containing pentobarbital regarding proper carcass disposal (FDA, 2003).

Factors capable of degrading sodium pentobarbital include exposure to air, heat, light (Branson, 2001), and indigenous microorganisms (Berryman et al., 2011). However, research has shown that pentobarbital can persist in the environment (Eckel et al., 1993; Peschka et al., 2006). Researchers recently discovered that sodium pentobarbital persisted in equine mortality compost piles up to 180 d (Cottle et al., 2010) and 224 d (Schwarz et al., 2013). The researchers identified a need for controlled experimentation utilizing replicated treatment and control groups to further investigate the fate of sodium pentobarbital in the environment. The objectives of this experiment were to observe the effects of proper composting on euthanized horse carcass degradation and sodium pentobarbital concentration in compost material along with the relative impact on environmental risk up to 367 d.

MATERIALS AND METHODS

The experiment was conducted at the Oklahoma State University Eastern Research Station located in Haskell, OK. Six separate compost bins were constructed on pastureland. Soils series were primarily Dennis-Verdigris complex (Dennis: 35%, Verdigris: 25%, minor components: 40%) and Parsons silt loam (Mollic Albaqualfs). One treatment and 1 control group were established using a randomized complete block design with 3 replicates for each group. Each compost bin measured 3.7 m^2 at the base and was constructed by forming a circle using two 6.1 m × 1.2 m metal horse panels supported by 3 steel t-posts (Fig. 1). The carbonaceous material, or bulking agent, for construction of compost piles consisted of hardwood chips mixed with yard waste (bulk density: 271 kg/m^{-3}) that were wetted to approximately 50% moisture content. Bulking agent was added to each bin at a depth of 0.46 m creating the pad. Twenty-four whiffle balls prefilled with bulking agent were centrally placed on each pad. Nylon hay twine was tied to each whiffle ball for retrieval at selected sampling times.

A licensed veterinarian provided 6 horse carcasses for use in the experiment. These horses had required euthanasia for health reasons. All horses were weighed and then sedated with an intravenous injection of 8 mL of xylazine. After sedation, the 3 horses in the treatment group were euthanized by intravenous injection of 60 mL Beuthanasia-D (Schering-Plough Animal Health, Union, NJ) containing 390 mg/mL of pentobarbital sodium. The 3 control group horses were anesthetized by intravenous injection of 15 mL of ketamine hydrochloride and then humanely euthanized by precise gunshot to the temporal lobe.

Following euthanasia, a carcass was placed on each pad, directly on the 24 whiffle balls that were evenly distributed between the fore- and hindquarters. The carcass remained at least 0.6 m from the bin walls. Nylon hay twine was used to secure the head and legs near the center of each carcass. Three serum samples and 1 liver sample were collected from each carcass for sodium

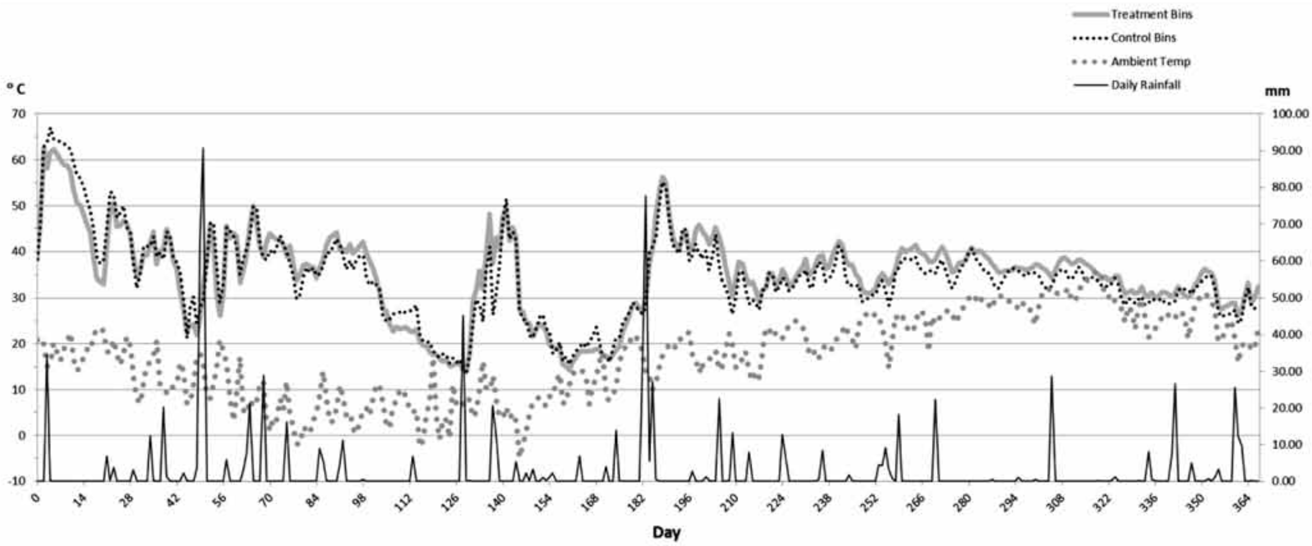

Figure 2. Average daily ambient air temperature and core compost pile temperature recorded by data loggers for sodium pentobarbital treatment and control groups along with daily rainfall amounts.

pentobarbital analysis before composting. Each serum sample was drawn from the contra lateral jugular and was placed into a 7 mL Monoject red stopper blood collection tube (Covidien, Mansfield, MA). A midsection of the liver weighing approximately 100 g was removed from each carcass and placed in sealed plastic bags. All samples were placed on ice and transported to the laboratory. Three HOBO U12 data loggers (Onset, Inc., Bourne, MA) with 4 temperature probes each (2 per bin) were set up to record hourly core compost pile temperatures. One temperature probe was placed directly on the carcass forequarter while 1 probe was placed directly on the carcass hindquarter, defining the core location. Hourly temperature readings were calculated as the average of fore- and hindquarter hourly readings. Daily temperature readings were calculated as the average of 24 hourly temperature readings. Each carcass was then surrounded with approximately 0.6 m of additional wood chips. Ambient air temperature and daily rainfall amounts were recorded by a weather station located on the Research Station grounds. Temperature and rainfall data were plotted to determine if composting was occurring as expected and to compare ambient temperature and rainfall to core compost pile temperatures.

Three composite soil samples were taken using a soil probe within each bin and analyzed for sodium pentobarbital on d -1 and 367. Each soil sample consisted of 15 subsamples that were collected to a 15 cm depth, thoroughly mixed and pooled in a sealed plastic bag. Three whiffle balls were collected from each bin on d 7, 14, 28, 56, 84, 129, 233, and 367 of composting and analyzed for pentobarbital concentrations. On d 129, each pile was opened and examined for bone and tissue remains. Carcass degradation rates were scored based on a scoring system developed by Brown (2007; Table 1). Each pile was then turned using a tractor with a front-end loader. Data loggers, carcass remains, and the remaining whiffle balls were repositioned within the core of each pile and covered with 0.6 m of wood chips.

Samples were shipped to the Illinois Department of Agriculture Animal Disease Laboratory (Centralia, IL) for sodium pentobarbital analysis using gas chromatography–mass spectrometry (GC–MS) procedures adapted and described by Adam and Reeves (1998). The equipment used in the analysis were a Hewlett-Packard (**HP**) 6890 GC, an HP 5973 mass selective detector (**MSD**), and an HP 7683 autosampler (Hewlett-Packard, Andover, MA). The minimum detection limit for pentobarbital was 0.005 mg/L and 0.005 mg/kg.

All data were subjected to analysis of variance with the use of the PROC GLM procedure in SAS version 9.1 (SAS Inst. Inc., Cary, NC) at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Average daily core compost pile temperature comparisons between pentobarbital treatment bins and control bins along with average daily ambient temperature and rainfall are illustrated in Fig. 2. The compost bin 367 d temperature range and mean for the treatment group, control group and ambient air temperature were (13.91 to 62.53; 34.97°C), (13.84 to 67.03; 34.18°C), and (−4.88 to 35.49; 17.18°C), respectively. Compost piles in treatment and control groups reached thermophilic temperatures (> 45°C) within 1 d and dropped to mesophilic temperatures (< 45°C) on d 16 and 17, respectively. However, thermophilic temperatures were

Table 1. Carcass degradation scoring system developed by Brown (2007)

Score	Description
1	Large amounts of flesh, hide and hair present. Internal fluid still visible. Carcass still discernible.
2	Flesh, hide and hair still present in smaller amounts. Carcass no longer discernible. No internal fluid visible.
3	Slight amounts of hair and hide present. Numerous large and small bones present.
4	No hide present. Minimal hair visible. Flesh completely degraded and only large bones present.
5	No flesh, hide, or hair present. Few to no large brittle bones present.

Table 2. Carcass degradation scores on d 129 and 367

Group	Horse weight, kg	Score, d 129	Score, d 367
Treatment	400	4	4
Treatment	558	3	4
Treatment	465	3	4
Control	288	4	4
Control	416	4	4
Control	651	3	4

periodically reached throughout the experiment, which could be correlated to daily rainfall amounts exceeding 45 mm and compost pile turning on d 129.

Initial horse weights and carcass degradation scores on d 129 and d 367 are listed in Table 2. The 3 heaviest horses (465, 558, and 651kg) received a lower carcass degradation score on d 129 compared to lighter weight horses (288, 400, and 416 kg). It is expected that the larger horses would take longer to degrade compared to the smaller horses. However, by d 367, all horses received the same degradation score as visual observations for all carcasses confirmed minimal to no soft tissue remaining. The carcass degradation process during composting begins with an initial breakdown of carcass soft tissue by naturally present microorganisms that produce heat, carbon dioxide, ammonia, and volatile organic compounds as byproducts (Berge et al., 2009). Following soft tissue decomposition, thorough mixing of the bulking agent and carcass promotes a more homogenous blend of carbon and nitrogen for optimum composting. Microorganisms will eventually degrade the carcass leaving only a few remaining bones. This typical degradation process was observed during the current study resulting in no hide, minimal hair, flesh degraded, and only large bones present.

Sodium pentobarbital concentrations from compost samples based on dry sample weight are listed in Table 3. As expected, pentobarbital concentrations were not detected in the control group. Average concentrations in the treatment group ranged from 33.95 to 93.83 mg/kg. No significant differences in sodium pentobarbital concentrations were observed in compost samples over time within the treatment group. Sodium pentobarbital concentrations were detected up to d 367. Significant differences were observed between treatment and control groups, except for d 28 and 367 comparisons. High variability from samples within the treatment group may account for the lack of significant difference on d 28 and 367. Cottle et al. (2010) reported compost sodium pentobarbital concentrations between 0.008 and 3.16 mg/kg after a duration of 180 d from 8 carcasses of horses euthanized with varied dosages (50 to 90 mL) of a solution containing 390 mg/mL sodium pentobarbital. The lower values may be explained by the difference in sampling techniques; Cottle et al. (2010) relied on grab samples versus whiffle balls filled with wood chips placed uniformly underneath each carcass. Schwarz et al. (2013) reported compost sodium pentobarbital concentrations between 0.36 and 11.65 mg/kg from a carcass of a horse euthanized with 120 mL of a solution containing 390 mg/mL sodium pentobarbital. The researchers used similar sampling methods as described in the current experiment but for a shorter duration (161 d). The current experiment utilized a randomized complete block design with replicated treatment and control groups for a duration of 367 d, which distinguishes it from previous work.

Sodium pentobarbital concentrations from liver, serum, and compost samples reported "as-received," which is based on wet sample weight, are illustrated in Table 4. A significant difference was detected between treatment and control groups for both liver and serum samples that were taken immediately after euthanasia, but before composting. The blood serum sample had a higher concentration as compared to the liver sample, 140.10 mg/L and 54.03 mg/kg, respectively. Schwarz et al. (2013) reported

Table 3. Sodium pentobarbital sample concentrations in compost samples from equine mortality static compost piles based on dry sample weight

	Sodium pentobarbital concentration (dry wt, mg/kg)									
	Compost sample									
Group	Day 7	Day 14	Day 28	Day 56	Day 84	Day 129	Day 233	Day 367	Significance level	SEM
Treatment	65.69	65.34	35.32	59.83	47.06	74.43	93.83	33.95	0.591	22.64
Control	0	0	0	0	0	0	0	0	1.0	0
Significance level	0.004	0.005	0.121	0.009	0.040	0.001	< 0.0001	0.136	< 0.0001[1]	16.01

[1]P-value across compost sample treatments and sampling times. Sample (n = 3).

Table 4. Sodium pentobarbital sample concentrations in compost samples from equine mortality static compost piles reported "as received"

	Sodium pentobarbital concentration (as received mg/kg)											
	Tissue		Compost									
Group	Liver, mg/kg	Serum, mg/L	Day 7	Day 14	Day 28	Day 56	Day 84	Day 129	Day 233	Day 367	Significance level	SEM
Treatment	54.03	140.10	33.40	35.78	22.49	48.65	35.85	46.48	77.52	25.15	0.416	17.14
Control	0	0	0	0	0	0	0	0	0	0	1.0	0
Significance level	0.0003	< 0.0001	0.053	0.04	0.192	0.005	0.038	0.008	< 0.0001	0.145	< 0.0001[1]	12.12

[1]*P*-value across compost sample treatments and sampling times. Sample ($n = 3$).

a sodium pentobarbital concentration of 92.6 mg/kg in horse liver immediately following euthanasia.

Carcass tissue containing sodium pentobarbital has been linked to secondary toxicosis when consumed by domestic pets (Edgson and Payne, 1967; Polley and Weaver, 1977; Reid, 1978; Anderson et al., 1979; Humphreys et al., 1980; Fucci et al., 1986), large exotic cats (Verster et al., 1990; Jurczynski and Zittlau, 2007), and other wildlife including bald eagles (Otten, 2001; Krueger and Krueger, 2002; O'Rourke, 2002). Kaiser et al. (2010) found sodium pentobarbital concentrations in tissue ranging from <20 to 4,084 mg/kg "as-received" from a euthanized horse carcass that had been partially buried in a ravine for over 2 yr. The same carcass was linked to secondary toxicosis in 2 dogs that consumed carcass tissue resulting in the death of 1 dog. According to the researchers, the estimated dosage was well over 100 mg/kg, assuming the dogs consumed 2.5% of their body weight. Fucci et al. (1986) reported a condition of light general anesthesia in a 28 kg dog that had consumed a dosage of approximately 14.5 mg/kg of pentobarbital.

Sodium pentobarbital found at the concentration described in this experiment is unlikely to cause secondary toxicosis even if the carcass tissue was exposed and consumed by dogs. For example, the oral lethal dose for dogs has been reported to be 85 mg/kg (Kaiser et al., 2010), while the anesthetic dose is 28–30 mg/kg (Branson, 2001). Assuming a liver concentration of 54.03 mg/kg pentobarbital, a 20-kg dog would have to consume approximately 32 kg of liver to reach a lethal dose and 10 kg to reach an anesthetic dose. The same dog would need to consume approximately 5 kg to achieve the light general anesthetic dose of 14.5 mg/kg reported by Fucci et al. (1986). In the current experiment, these scenarios are unlikely to occur as a 20-kg dog would only ingest approximately 0.5 kg, since dogs generally consume 2.5% of their body weight daily (Hoskins, 1997). Furthermore, by properly enveloping the carcass with wood chips and constructing a barrier, the carcass would be protected from scavenging animals. Moreover, carcass degradation followed by homogenous compost mixing and land application as a soil amendment allows for significant pentobarbital dilution.

During livestock composting, the bulking agent acts as a biofilter between the carcass and the environment by trapping most of the leachate and odors. In this experiment, sodium pentobarbital was detected at 0.2 mg/kg (dry wt.) in soil samples collected from each treatment bin on d 367 (Table 5) indicating that the drug leached from the carcass through the 0.46 m compost pad and into the soil. This may have been due to the use of woodchips, which are a coarser material compared to wood shavings, as the bulking agent. However, Cottle (2011) also observed sodium pentobarbital leaching in laboratory-designed composting columns containing livers from euthanized equine carcasses mixed with horse manure, wood shavings, and waste hay.

Conclusions

Composting carcasses of horses euthanized with sodium pentobarbital successfully degraded soft tissue but showed no clear trend of reducing drug residues. When using a bulking agent such as hardwood chips, sodium pentobarbital can leach from the carcass, through the compost pad, and into the soil. Future research could investigate the impact of other bulking agents or impervious surfaces underneath the compost pile on sodium pentobarbital movement. These findings confirm the persistence of sodium pentobarbital from equine mortality compost piles and emphasize the importance of proper carcass management of animals

Table 5. Sodium pentobarbital sample concentration in composite soil samples below equine mortality static compost piles reported as dry weight

	Sodium pentobarbital concentration (dry wt. mg/kg)			
	Soil			
Group	Day -1	Day 367	Significance level	SEM
Treatment	0	0.20	0.0002	0.03
Control	0	0	1.0	0
Significance level	1.0	< 0.0001	< 0.0001[1]	0.02

[1]*P*-value across compost sample treatments and sampling times. Sample ($n = 3$).

euthanized with a barbiturate to reduce environmental impact and secondary toxicosis.

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