

Fate of sodium pentobarbital in rendered products

John J. O'Connor, DVM, MPH; Clarence M. Stowe, VMD, PhD; Robert R. Robinson, BVSc, MPH, PhD

SUMMARY

The fate of pentobarbital through rendering was evaluated by following a group of euthanatized animals through a commercial rendering facility. Samples of material were collected at various points in the rendering process, and assays for pentobarbital were conducted by an ultraviolet spectrophotometric method. The results indicated that the pentobarbital, or a closely related analogue, survived rendering without undergoing degradation. The pentobarbital was distributed approximately equally between the meat and bone meal and tallow fractions. If pentobarbital-euthanatized animals are processed along with other raw materials throughout a day's production, the likelihood of significant residues being present in rendered products is minimal.

The contamination of animal feeds with hazardous substances (drugs, pesticides, heavy metals, and industrial chemicals) poses a potential threat to the safety of animals and, ultimately, persons. The rendering industry produces a number of products, such as meat and bone meal and fats, that are frequently used as components of animal feeds. One concern is the practice of rendering dead animals whose death may have been due to, or associated with, exposure to a toxic substance.

Euthanatized animals which are rendered may contain hazardous substances. Although there are several drug and nondrug methods of euthanasia (decompression, nitrogen gas, and carbon monoxide chambers), the AVMA Panel on Euthanasia¹ rated the IV injection of a barbituric acid derivative as the preferred method for individual dogs, cats, and other small animals. Barbiturates are considered ideal for small animals because of the rapid and smooth induction of anesthesia and minimal discomfort to the animal. They are also occasionally used for this purpose in large animals. The most common barbiturate used for euthanasia is sodium pentobarbital.

In metropolitan areas, humane societies and municipal animal control facilities euthanatize a large number of dogs and cats each year. For example, the Department of Animal Regulation for the City of Los Angeles euthanatized 52,216 animals with sodium pentobarbital during

the 1982-1983 fiscal year.^a In Los Angeles County, 76,375 animals were euthanatized, the vast majority with pentobarbital, during the same period.^b Animals from both districts were disposed of by rendering. In New York City, approximately 56,000 animals were euthanatized in 1983 with pentobarbital and rendered at a plant in New Jersey.^c It is roughly estimated that the disposal of euthanatized animals by municipal animal control facilities is approximately 40% by rendering, 40% by burial, and 20% by cremation. In larger metropolitan areas, the percentage of rendering for disposal is likely to be higher, primarily for environmental and economic reasons.^d

There have been a number of reports, primarily from England, of relay or secondary toxicosis, including deaths, in animals that have ingested tissues of other animals euthanatized with pentobarbital.²⁻⁷ The instances reported involved dogs or cats that had accidentally ingested tissues or were fed meat from cattle or horses which had been euthanatized with pentobarbital. In 2 of the reports, dogs became comatose after ingesting meat from euthanatized animals, even though the meat had apparently been cooked.^{2,3} In one report in which a dog exhibited pentobarbital toxicosis after ingesting the thoracic organs of a euthanatized calf, the pentobarbital concentration in the calf kidney did not decrease after boiling for 20 minutes.⁴

There have been no reports that rendered products processed from pentobarbital-euthanatized animals caused any deleterious effects in animals that consumed feeds made from such products. However, it is unlikely that pentobarbital contamination of a feed ingredient would have been considered as a possible cause of any observed adverse effects. The present study was conducted to determine whether sodium pentobarbital survived the heat processing of rendering and, if so, to determine its relative distribution and concentration in the finished rendered products, ie, meat and bone meal and fat (tallow).

Materials and Methods

A large commercial animal control facility operating in the Minneapolis/St Paul metropolitan area agreed to separate for 1 week those animals euthanatized with sodium pentobarbital from other animals collected by the facility, but which had died from other causes. The number and species of animals, the estimated weight of each animal, and the total amount of sodium pentobarbital solution used were recorded (49 dogs, 32 cats, and 1 sea gull, collectively weighing approx 665 kg). A total of 104 g of sodium pentobarbital was used. The facilities of a commercial "batch type" rendering operation were made available for this study.

^a Ziegler W, Department of Animal Regulation, Los Angeles, Calif: Personal communication, 1984.

^b Baca G, Los Angeles County Department of Animal Care and Control, Los Angeles, Calif: Personal communication, 1984.

^c Monteferrante D, American Society for the Prevention of Cruelty to Animals, New York, NY: Personal communication, 1984.

^d Arkow P, The National Animal Control Association: Personal communication, 1984.

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From the Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD 20857 (O'Connor); the Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St Paul, MN 55108 (Stowe, Robinson); and the Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, MN 55455-0318 (Robinson).

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Euthanized animals were unloaded and mechanically shoveled into a loading auger as the first material to be processed in a day's operation. The material was entered a "prebreaker" which ground the carcasses into pieces of approximately 2 inches or less. The material was then augered into a loading tank of the same capacity as the cookers (about 4,500 kg). The raw material selected to complete the fill for the first cooker consisted of pork and beef offal from local slaughterhouses. Tallow (approx 130 kg) was added to the loading tank to reduce cooking time. A small amount of a commercial antioxidant and an antifoaming agent were also added.

This load was cooked under continuous agitation in a steam-jacketed cooker (No. 9) until the moisture content was reduced to a predetermined level (about 3 hours at 127 to 132 C). The contents were released onto a percolator which allowed the free fat to drain from the tankage (solids). The tankage was transported to a screwpress expeller which extracted additional fat from the tankage under high pressure. The cake, or "pressed cracklings," from the expeller was then ground and passed over screens to produce the finished meat and bone meal product. The free fat from the percolator drain pan and fat extracted by the expeller were centrifuged to remove solids before entering the final holding tank.

Samples were taken for pentobarbital determination from cooker No. 9 at the beginning and end of unloading. Pressed cracklings (meat and bone meal cake) and pressed fat were taken when material from cooker No. 9 left the expeller. Comingled fat, consisting in part of fat from cooker No. 9, was also collected after centrifugation when it entered the final holding tank. Comparable control samples were taken from a separate cooker that did not contain euthanized animals.

The extraction and ultraviolet spectrophotometric techniques used in the analysis were variations on procedures described for the "optical density difference" method of Goldbaum and others.⁸⁻¹²

Procedure—For material other than fat, a 5-g sample was blended with 25 to 30 ml of acetate buffer (pH 5.5; 59 ml of 0.1N acetic acid added to 141 ml of 0.1N sodium acetate) that had been warmed to facilitate homogenization. The suspension was then extracted with 100 ml of chloroform. The chloroform fraction was filtered, and 80 ml of the extract was shaken with 5 ml of 0.45N sodium hydroxide. The sodium hydroxide layer was removed and centrifuged. A 3-ml aliquot of this solution was diluted 1:1 with 0.45N sodium hydroxide, and the solution was divided into two 3-ml portions. A 0.5-ml amount of 2.25M sodium dihydrogen phosphate was added to one portion to lower the pH to 10.5, and an equal volume of 0.45N sodium hydroxide was added to the other sample to equalize volumes. The solutions were added to 2 microcuvettes, both volumes were placed in the spectrophotometer, and the absorbance was determined from 300 to 230 nm.

For fat samples, a 5-g amount was extracted directly with 100 ml of chloroform. The rest of the procedure was identical with that for other samples, except that 0.9N sodium hydroxide and 4.5M sodium dihydrogen phosphate solutions were used to reduce emulsion formation.

Results

The estimated amount of raw material in cooker No. 9 at the beginning of the process was 4,312 kg, including euthanized animals plus additional fill of meat and bone scraps from packing plants. Assuming 55% moisture loss during cooking, 1,943 kg remained, and assuming no degradation of sodium pentobarbital, theoretically 53.7 mg/kg should have been present in the final product as it left the cooker. The actual results were 60.6 mg/kg (mean of samples taken at the beginning and end of unloading the

cooker), 40.5 mg/kg in the crackling, 40.9 mg/kg in the pressed fat, and 21.1 mg/kg in the comingled fat. Sodium pentobarbital was not detected in similar samples from other cookers during that day's processing.

Discussion

The close agreement between estimated and detected levels of sodium pentobarbital would indicate that virtually no degradation of the drug occurred during this conventional rendering process. By the time material from cooker No. 9 had reached the expeller, there had been some mixing with material from other cookers, which would account for the lower levels of drug detected in crackling and pressed fat.

The extent to which the pentobarbital would be distributed between the meat and bone meal or fat fractions would depend on a number of interacting factors including the lipid solubility of pentobarbital, protein-binding capacity, and the extent of ionization. The pentobarbital levels determined for crackling (40.5 mg/kg) and pressed fat (40.9 mg/kg) indicate that the drug was equally distributed between the 2 fractions. This is consistent with a tissue distribution study in the dog, in which the concentration of pentobarbital in various tissues 3 hours after IV administration was found to be about the same or somewhat higher than that in plasma with no extensive localization in fat.¹³ The extent to which pentobarbital binds to serum protein has been variously reported to be 35% and 50%.^{14,15} An in vitro study to measure the partition ratio of pentobarbital between buffer at pH 7.4 and peanut oil revealed that 50% of the pentobarbital was in the oil phase.¹³ It therefore seems that pentobarbital would generally be equally distributed between the 2 fractions during rendering.

The concentration of pentobarbital that could occur in rendered products under commercial rendering practices would depend on the dosages of pentobarbital used for euthanasia, the ratio of euthanized animals to other raw materials processed, the extent of prior mixing of raw materials, and the subsequent dilution during processing. At the facility where this study was done, the euthanized animals would normally have been distributed among several cookers for processing to produce a product of uniform quality. The pentobarbital-contaminated material from cooker No. 9 represented 5% of the material processed that day in the entire plant. The concentration of pentobarbital in the meat and bone meal or tallow produced for the day would have been approximately 3 mg/kg, assuming there had been thorough mixing of the raw materials and uniform dilution of pentobarbital in the finished products. If the euthanized animals were rendered separately, the final pentobarbital concentration of a meat and bone meal or tallow fraction so produced would be approximately 350 mg/kg. However, this would not normally be done by commercial renderers, since the high concentration of hair would reduce the quality of the meat and bone meal produced.

Meat and bone meal is used as a source of protein and other nutrients in the diets of poultry and swine and in pet foods, with lesser amounts used in the feed of cattle and sheep. Animal fat is also used in animal feeds as an energy source.

The degree of animal exposure to pentobarbital through

TABLE 1—Theoretical consumption levels of pentobarbital by young swine and chicks

Pentobarbital conc (mg/kg) in meat and bone meal and fat (equal conc in both)	Pentobarbital conc (mg/kg) in final ration (9% meat and bone meal, 6% fat)	Pentobarbital consumed (mg/kg/day)*	
		Swine	Chicks
3	0.5	0.04	0.06
60	9.0	0.72	1.26
100	15.0	1.20	2.10
350	52.5	4.20	7.35

* Milligram of pentobarbital/kg of body weight/day = concentration in feed ingredients (mg/kg) × 0.15 (proportion of ingredients in total diet) × 0.08 or 0.14 (proportion of feed consumed by swine or chicks/kg of body weight/day).

Conc = concentration.

TABLE 2—Theoretical consumption levels of pentobarbital by young dogs

Pentobarbital conc (mg/kg) in meat and bone meal and fat (equal conc in both)	Pentobarbital conc (mg/kg) in final ration (20% meat and bone meal, 8% fat)	Pentobarbital consumed (mg/kg/day)*
60	16.8	1.31
100	28.0	2.18
350	98.0	7.64

* Milligram of pentobarbital/kg of body weight/day = concentration in feed ingredients (mg/kg) × 0.28 (proportion of ingredients in total diet) × 0.078 (proportion of body weight consumed in feed/day).

Conc = concentration.

the feed would depend on the pentobarbital concentration of the feed ingredients (meat and bone meal and/or fat), the proportion of the ingredient(s) used in the total diet, and the amount of the diet consumed by the animal. Up to 6% fat can be added successfully to the ration of livestock and poultry, although pelleted poultry diets may contain as much as 7% or 8% added fat.^{16,17} Since vegetable oils are usually more expensive, the fats predominantly used in animal feeds are of animal origin.¹⁶ Meat and bone meal can be used in poultry feeds at levels up to 8% or 9% of the ration.¹⁷ A similar level of meat and bone meal could be used in swine rations.¹⁸ Young growing animals and those in production would require higher levels of dietary protein and energy and, thus, would ingest relatively more pentobarbital than animals fed maintenance diets. Young swine consume about 8% of their body weight in feed per day, and chicks consume about 14%.¹⁹ Table 1 shows the amount of pentobarbital consumed by young swine and chicks fed a diet of 9% meat and bone meal and 6% fat and containing various hypothetical concentrations of pentobarbital in the contaminated feed ingredients.

Meat and bone meal and animal fat are standard ingredients in most commercial dry dog foods. A good-quality dry dog food would contain a meat and bone meal concentration of 14% to 20%. Fat is frequently added at a rate of from 4% to 8% of the ration.⁶ Table 2 shows the amount of pentobarbital consumed by a young dog that eats food at a rate of 7.8% of his body weight per day¹⁹ and consumes varying hypothetical levels of pentobarbital in the contaminated ingredients.

Possible biological consequences caused by the pres-

⁶ Morman R, Ralston Purina Co. Personal communication, 1984.

ence of pentobarbital in animal feeds are as follows: (1) The oral and IV sedative dosage of sodium pentobarbital for most species is approximately 4.4 mg/kg of body weight.²⁰ If young chickens or dogs were exposed to diets containing meat and bone meal and fat contaminated with pentobarbital at the level detected in material from cooker No. 9 (60 mg/kg), these animals would be consuming approximately a fourth of the sedative dose of pentobarbital. (2) Barbiturates are well known for their ability to stimulate the activity of drug-metabolizing enzymes in liver microsomes. Since induction causes a nonspecific increase in the concentration of microsomal enzymes,²¹ there is low substrate specificity. Consequently, the metabolism of a large variety of endogenous and exogenous substances could be affected and could lead to alterations in the biological activity of numerous compounds. The level and duration of exposure necessary to cause enzyme induction in domestic animals would be difficult to predict for pentobarbital, since most of the investigations in this area have been done with the model inducing agent, phenobarbital.

Factors affecting the possibility of residues of pentobarbital occurring in tissues of slaughtered livestock include the degree of exposure from the diet, its tissue distribution, and its rate of metabolism and excretion. Although pentobarbital is lipid-soluble, it seems to be equally distributed in body tissues. The rate of metabolism of barbiturates varies considerably between and among various species. Pentobarbital is metabolized at a rate of 15%/hour in the dog, whereas in sheep, the rate is 49%/hour.²⁰ Repeated exposure at a level sufficient to cause enzyme induction would accelerate the rate of metabolism and elimination.

The present study demonstrated that pentobarbital, or a close analogue, survived the rendering process. However, a number of factors previously discussed would tend to minimize the likelihood of any significant biological effects, either for animals consuming contaminated products or ultimately, for the human consumer. These factors include the dilution of pentobarbital-contaminated material with other raw materials during rendering, the lack of a tendency for pentobarbital to concentrate in either the tallow or protein fractions, further dilution with other feed ingredients in formulating a complete ration (Tables 1 and 2), and pentobarbital's rate of metabolism in domestic animals. Although the potential for any biological effects seems small, additional information is needed on those facilities that process pentobarbital-euthanized animals to determine more conclusively the risks, if any, posed by the processing of such material.

Finally, the potential of other chemical contaminants (eg, heavy metals, pesticides, and environmental toxicants, which may cause massive herd mortalities) to degrade during conventional rendering needs further evaluation, so that clear guidelines can be established.

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