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Fatal bromethalin intoxication in 3 cats and 2 dogs with minimal or no histologic central nervous system spongiform change

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Abstract. Use of the neurotoxic rodenticide bromethalin has steadily increased since 2011, resulting in an increased incidence of bromethalin intoxications in pets. Presumptive diagnosis of bromethalin toxicosis relies on history of possible rodenticide exposure coupled with compatible neurologic signs or sudden death, and postmortem examination findings that eliminate other causes of death. Diagnosis is confirmed by detecting the metabolite desmethylbromethalin (DMB) in tissues. In experimental models, spongiform change in white matter of the central nervous system (CNS) is the hallmark histologic feature of bromethalin poisoning. We describe fatal bromethalin intoxication in 3 cats and 2 dogs with equivocal or no CNS white matter spongiform change, illustrating that the lesions described in models can be absent in clinical cases of bromethalin intoxication. Cases with history and clinical signs compatible with bromethalin intoxication warrant tissue analysis for DMB even when CNS lesions are not evident.

Key words: Bromethalin; cats; dogs; intoxication; neurotoxic; rodenticide; spongiform change.

The neurotoxic rodenticide bromethalin has become increasingly popular since 2011 when Environmental Protection Agency regulations went into effect restricting use of 2nd-generation anticoagulant rodenticides (Brutlag AG, et al. Pet poisonings involving new, EPA-approved bromethalin rodenticides: implications for pets and humans [abstract]. Clin Toxicol 2013;51:711). Bromethalin is the active ingredient in many commercial rat and mouse baits (0.01% blocks, pellets, and place packs) and worm-shaped mole baits (0.025%). Bromethalin targets the central nervous system (CNS) and is associated with 2 types of presentation: a convulsant syndrome characterized by muscle tremors, hyperexcitability, hyperthermia, and seizures; and a progressive paralytic syndrome involving ascending ataxia and/or paresis, proprioceptive deficits, and CNS depression.[11] Severely affected animals develop diminished or absent deep pain response, ascending paralysis, and stupor or coma. High dosages cause rapid onset of signs; lower dosages cause delayed onset of 48 h or longer. In dogs, higher dosages often result in the convulsant syndrome, whereas lower dosages usually cause the paralytic syndrome.[5] Most cats develop the paralytic syndrome regardless of dosage.[4] Although the reported minimum lethal dose in dogs is 2.5 mg/kg,[3] the ASPCA Animal Poison Control Center (APCC; Urbana, IL) case records include a fatality in a dog ingesting 0.95 mg/kg bromethalin.[7] In cats, the reported minimum lethal dose is 0.45 mg/kg.[4] APCC case records have documented clinical signs of toxicosis in cats ingesting 0.24 mg/kg bromethalin.[7]

A review of the Pet Poison Helpline (Bloomington, MN) database showed a 33% increase in bromethalin-related calls between 2011 and 2012 (Brutlag et al. 2013). Yet a recent literature review found only 4 published case reports of bromethalin poisoning: a dog, a cat, a raccoon, and a human.[1,2,9,10] Most information published on bromethalin intoxication relies on administration studies to describe postmortem lesions. In these models, dogs were administered 6.25 mg/kg and cats were administered 1.5 mg/kg bromethalin, which is more than twice the respective minimum lethal dosages.[5,6] The characteristic histologic lesion in bromethalin-dosed animals was diffuse vacuolar spongiosis of the white matter in the cerebellum, cerebrum, brainstem, spinal cord, and optic nerve.

We describe 5 confirmed cases (3 cats and 2 dogs) of bromethalin poisoning between 2011 and 2016 diagnosed at the University of Kentucky Veterinary Diagnostic Laboratory. All cases had histories and clinical signs consistent with bromethalin intoxication. Although CNS white matter lacked

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definitive spongy degeneration, bromethalin exposure was confirmed in all cases by tissue analysis by the California Animal Health and Food Safety Laboratory System.

Tissue samples were extracted using ethyl acetate (Thermo Fisher Scientific, Waltham, MA), concentrated, and analyzed by reverse-phase ultrahigh-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS; 6460 LC-MS system, Agilent, Santa Clara, CA). Analysis was performed using electrospray ionization in negative ion mode. Appropriate negative and positive control tissue samples were analyzed with each batch of diagnostic samples.[8] Bromethalin is rapidly absorbed after ingestion and metabolized via N-demethylation[11] in the liver to desmethylbromethalin (DMB) in most species, making DMB a suitable analyte to confirm bromethalin exposure.[8] Additionally, analyzing for DMB confirms ingestion and metabolism of the parent compound. This method is typically used to provide qualitative (positive or negative) results.

Case 1

A 5-y-old spayed female Domestic Shorthair barn cat was obtained by her owners 3 y previously from an animal shelter. The cat was vaccinated (unspecified vaccines) at the time of adoption but not since. The owner fed the cat commercial dry cat food and any dead mice that he found. The cat roamed neighboring farms where rodenticide exposure was possible but not confirmed. Initially the owner noticed that the cat was staggering and acting strangely. She bit the owner, which was unusual given that she was normally very friendly. On presentation to the clinic, the cat was ataxic but could stand. She had proprioceptive deficits in all 4 limbs, worse in the hindlimbs. She displayed superficial and deep pain reflexes. No pain response was elicited on palpation of her limbs or spine. The owner declined testing and treatment and took the cat home. Two days later, the cat was presented to the clinic in lateral recumbency, with bilateral hindlimb paralysis, both forelimbs curled inwards, and slight muscle fasciculations. Reflexes were absent in both hindlimbs, with the exception of a slight deep pain response. The cat appeared blind, with no pupillary light response. Her mentation was stuporous, almost comatose, with an exaggerated bite reflex. The owners elected euthanasia.

On postmortem examination, the cat was in good body condition with good postmortem preservation. No gross anatomic lesions were observed. A rabies test by direct fluorescent antibody (FA) testing was negative. Histologically, there was mild, focal, subacute lymphoplasmacytic enteritis with tapeworms present. No lesions were observed in the brain, heart, lung, liver, kidney, spleen, stomach, large intestine, or pancreas. Fecal flotation revealed moderate numbers of *Toxocara cati* eggs. Liver and kidney samples were analyzed for lead by inductively coupled plasma–mass spectrometry (ICP-MS), and lead was not detected. DMB was detected in the perirenal fat and kidney; trace amounts were also detected in the brain and liver.

Case 2

A young adult female feral cat was presented to a local animal shelter with hindlimb paralysis. Four other cats in the same colony were similarly affected, suggesting malicious poisoning. The cat died shortly after admission and was presented for postmortem examination. She was in fair body condition and good preservation, although partially frozen. The bladder was distended, but otherwise normal grossly; the urethra was patent, and urine was expressible. Rabies virus, feline infectious peritonitis virus, and panleukopenia virus FA tests were negative. Aerobic cultures of liver, lung, and colon were negative for pathogens.

Histologically, multiple tissues including brain had moderate-to-severe freezing artifact. Some areas of the cerebrum had a spongiotic appearance. Scattered protozoal cysts were within the myocardium. The spleen was contracted with moderate hypocellularity of the red pulp. Several airways contained fluid, and the intestines contained parasite ova and tangential sections of cestodes. No significant histologic lesions were evident in the liver, kidney, or pancreas. Perirenal fat tested positive for DMB.

Case 3

A 2-y-old spayed female Domestic Longhair cat was presented to the veterinary clinic with severe ataxia and proprioceptive deficits in the hindlimbs. The owner had found bromethalin rat poison in the cat's environment. Initially, the cat could sit up, was alert, and had a good appetite. Withdrawal reflexes and femoral pulses were normal. All vaccinations, including rabies vaccination, were current. Complete blood count and serum chemistry panel results were within normal reference intervals. Spinal radiographs revealed no abnormalities. Treatment consisted of intravenous fluids, dexamethasone, and tetracycline as well as subcutaneous thiamine. The cat deteriorated, becoming depressed and anorectic. She died after 2 d and was presented for postmortem examination.

Grossly, the carcass was in good preservation. Mucoid material filled the right tympanic bulla. The descending colon was packed with fecal material, and the stomach was empty. Rabies virus FA was not performed. Feline infectious peritonitis virus FA was negative. Aerobic cultures of the liver, lung, colon, and ear swab were negative for pathogens. Histologically, the lumen of the tympanic bulla contained large amounts of acidophilic proteinaceous material, moderate numbers of neutrophils, and low numbers of macrophages. The adjacent fibrovascular tissue contained similar inflammatory cells, lymphocytes, and hemosiderophages.

Table 1. Estimated desmethylbromethalin (DMB) tissue concentrations in case 4.

Tissue	Estimated DMB concentration (µg/kg wet weight)
Fat	390
Kidney	1.20
Brain	0.26
Liver	0.041

The liver had mild generalized hepatocellular vacuolation (lipid and glycogen types). No significant lesions were evident in the brain; cervical, thoracic, or lumbar spinal cord; heart, lung, kidney, spleen, pancreas, small intestine, or large intestine. Brain was the only tissue available for testing and was positive for DMB.

Case 4

A 9-wk-old female Beagle was found dead one morning, despite appearing healthy the night before. On postmortem examination, the carcass was in good preservation. Bright green fecal material matted the hindlimbs and perianal region. The stomach contained a small plastic pouch, bright green granular material, and ingesta. Green granular material was present throughout the intestines. The gastrointestinal (GI) mucosa was stained bright green. Lungs were congested and hemorrhagic. Rabies virus FA was not performed. Histologically, occasional blood vessels in the cerebral white matter were surrounded by minimal numbers of neutrophils, macrophages, and lymphocytes. Alveoli and upper airways contained low numbers of macrophages, eosinophils, and erythrocytes, as well as a moderate amount of proteinaceous material. Hepatic portal areas were expanded by eosinophils and macrophages. No significant lesions were evident in the kidney, small intestine, or large intestine. Liver was analyzed for anticoagulant rodenticides including brodifacoum, bromadiolone, chlorophacinone, coumachlor, difethialone, diphacinone, and warfarin using UPLC-MS/MS; none was detected. Stomach contents and green bait material were analyzed for organic compounds by gas chromatography–mass spectrometry. No toxic compounds detectable by this method were found. Analysis of the GI contents by ICP-MS was negative for arsenic, ruling out Paris green as the green substance. GI contents, liver, brain, kidney, and fat were analyzed for DMB, which was detected in all tissues (Table 1). To provide an estimate of the relative DMB levels in different tissue types, DMB chromatographic peak areas from the samples were compared to those from certified DMB standards. This quantification method has not been fully validated and therefore does not meet standards for accuracy or precision; nevertheless, it was useful to confirm which tissue types are ideal for DMB analysis. Perirenal fat contained the highest concentration of DMB, followed by the kidney.

Case 5

A 7-mo-old female mixed-breed dog was submitted for autopsy by its owner. The owner stated that the dog had been exposed to rat poison and had shown unspecified neurologic signs. Attempts to obtain veterinary records and further history were unsuccessful. The carcass was moderately decomposed and had been frozen. No gross anatomic lesions were observed. Rabies virus FA was not performed. Histologically, the cerebellum and brainstem showed perivascular hemorrhages throughout the sections. Some areas of the cerebrum had a spongiform appearance. Alveolar septa were congested, and lumens contained proteinaceous, eosinophilic material. Most hepatocytes were vacuolated. No significant abnormalities were evident in the heart, kidney, urinary bladder, pancreas, thymus, small intestine, or diaphragm. Fecal flotation showed many *Ancylostoma* sp. and *Toxocara canis* ova. Aerobic cultures of liver, lung, small intestine, and colon were negative for pathogenic bacteria. Fat was positive for DMB.

In all of the cases described herein, the animals displayed clinical signs compatible with bromethalin intoxication. All had confirmed or possible access to rodenticide. Most significantly, DMB was detected in tissue samples from all of the cases. The most variable finding in these cases was the histologic appearance of the CNS white matter (Table 2). Three cases had no lesions and 2 had areas of spongy change with equivocal interpretation. Spongiform change can be attributable to postmortem autolysis, processing, or other artifact.[12] The 3 cases with short postmortem intervals (minimal autolysis) had no evidence of spongiform change in the brain. In contrast, both cases with areas of spongiform change had a 2–3-d postmortem interval and were submitted frozen; hence, the spongiform change is likely artifactual. Our retrospective review demonstrates that clinical cases of bromethalin intoxication can lack the classic CNS white matter spongiform change described in experimental models. One possibility is that dosages ingested in the clinical cases were much lower than in administration studies. White matter spongiform degeneration was not apparent at the lower dose in rats.[11] Also, the time from ingestion to death may have affected the histologic appearance of the white matter. The most pronounced spongy degeneration was found in a cat that survived 20 d after dosing.[6]

Bromethalin intoxication should be considered in cases with compatible clinical signs and history. Even if CNS white matter spongy change is not present, tissue analysis for DMB is warranted. Bromethalin and DMB are lipid soluble, based on their octanol–water partition coefficients (6.70 and 4.26, respectively), and they concentrate in fat, making fat the ideal tissue for analysis. If fat is unavailable, kidney may be the next best tissue to analyze.

Table 2. Case summaries of bromethalin-intoxication cases in the current study.

Case	Species	Neurologic signs	Rodenticide access	Histologic lesions in CNS	Tissues analyzed for DMB	DMB detected
1	Cat	Yes	Possible	No	Fat; kidney; brain; liver	Yes (all)
2	Cat	Yes	Possible	No	Fat	Yes
3	Cat	Yes	Yes	Equivocal	Brain	Yes
4	Dog	Uncertain	Yes	No	Fat; kidney; brain; liver	Yes (all)
5	Dog	Yes	Yes	Equivocal	Fat	Yes

CNS = central nervous system; DMB = desmethylbromethalin.

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References

1. Bates MC, et al. Atypical bromethalin intoxication in a dog: pathologic features and identification of an isomeric breakdown product. BMC Vet Res 2015;11:244.
2. Bautista AC, et al. Bromethalin poisoning in a raccoon (*Procyon lotor*): diagnostic considerations and relevance to nontarget wildlife. J Vet Diagn Invest 2014;26:154–157.
3. Dorman DC, et al. Bromethalin toxicosis in the dog. Part I: clinical effects. Part II: selected treatments for the toxic syndrome. J Am Anim Hosp Assoc 1990;26:589–598.
4. Dorman DC, et al. Bromethalin neurotoxicosis in the cat. Prog Vet Neurol 1990;1:189–196.
5. Dorman DC, et al. Diagnosis of bromethalin toxicosis in the dog. J Vet Diagn Invest 1990;2:123–128.
6. Dorman DC, et al. Neuropathologic findings of bromethalin toxicosis in the cat. Vet Pathol 1992;29:139–144.
7. Dunayer E. Bromethalin: the other rodenticide. Vet Med 2003;98:732–736.
8. Filigenzi MS, et al. Method for the detection of desmethylbromethalin in animal tissue samples for the determination of bromethalin exposure. J Agric Food Chem 2015;63:5146–5151.
9. Martin T, Johnson B. A suspected case of bromethalin toxicity in a domestic cat. Vet Hum Toxicol 1989;31:239–240.
10. Pasquale-Styles MA, et al. Fatal bromethalin poisoning. J Forensic Sci 2006;51:1154–1157.
11. van Lier RB, Cherry LD. The toxicity and mechanism of action of bromethalin: a new single-feeding rodenticide. Fundam Appl Toxicol 1988;11:664–672.
12. Wohlsein P, et al. Nonlesions, unusual cell types, and postmortem artifacts in the central nervous system of domestic animals. Vet Pathol 2013;50:122–143.