J Forensic Sci, September 2006, Vol. 51, No. 5
doi:10.1111/j.1556-4029.2006.00218.x
Available online at: www.blackwell-synergy.com

CASE REPORT

Melissa A. Pasquale-Styles,[1] *M.D.; Mark A. Sochaski,*[2] *Ph.D.; David C. Dorman,*[2] *D.V.M., Ph.D.; Willane S. Krell,*[3] *M.D.; Aashit K. Shah,*[4] *M.D.; and Carl J. Schmidt,*[5] *M.D.*

Fatal Bromethalin Poisoning

ABSTRACT: Bromethalin is a neurotoxin found in some rodenticides. A delusional 21-year-old male presented to a hospital with altered mental status the day after ingesting a bromethalin-based rodenticide. He died 7 days after his self-reported exposure to *c*. 17 mg bromethalin (equivalent to 0.33 mg bromethalin/kg). His clinicopathologic course was characterized by altered mental status, obtundation, increased cerebrospinal fluid pressure, cerebral edema, death, and diffuse histologic vacuolization of the white matter in the central nervous system seen on microscopic examination at autopsy. The presence of a demethylated form of bromethalin in the patient's liver and brain was confirmed by gas chromatography with mass spectrometry. Clinical signs and lesions observed in this patient are similar to those seen in animals poisoned with bromethalin. This case illustrates the potential for bromethalin ingestion to result in fatal human poisoning.

KEYWORDS: forensic science, bromethalin, rodenticide, rat poison, neurotoxicity syndromes, analytical chemistry

Various types of rodenticides including first- and second-generation anticoagulants, cholecalciferol, zinc phosphide, strychnine, and bromethalin have been used to control mice and rat populations. Although bromethalin accounts for a significant number of accidental rodenticide poisonings in veterinary medicine, human exposures are unusual. In 2004, 643 bromethalin exposures were reported to 62 major poison control centers across the United States (1). In contrast, nearly 16,400 anticoagulant rodenticide exposures were reported during the same time. The bromethalin exposure doses were not reported, but the vast majority of exposed people experienced no signs of toxicity. Only three people had "major" effects, which were described simply as either "life-threatening" or "associated with significant disability." The clinical or pathologic findings in human poisoning have never been described in detail in the English literature, and, to our knowledge, a fatal human poisoning has not been previously reported.

Case Report

A 21-year-old black male was transported to the emergency room after his family found him with multiple open packages of rat poison "place packs," and he displayed increasingly odd behavior consisting of repetitive speech, labile emotions, and diminished responsiveness to direct questioning. He told the transporting rescue personnel that he had eaten "8 packs of No Pest® (United Industries Corporation, St. Louis, MO) rat and mice killer and a box of Ex-Lax® (Novartis AG, Basel, Switzerland)" the night before. In the emergency room, he claimed to have taken rat poison "to kill the rats inside" and "to kill the rat genes." He had normal vital signs but was alert and oriented to person and place only. The rest of his physical examination was unremarkable. An electrocardiogram was essentially normal. A urine drug screen was positive for barbiturates. He experienced one episode of emesis in the emergency room and was treated with promethazine, thiamine, folic acid, and diphenhydramine. He was said to be "medically cleared," and a provisional diagnosis of major depression with psychosis was made. He was transferred from the emergency room to an inpatient psychiatric treatment center at a different location.

He was in the psychiatric hospital for 3 days and was reported to be "obtunded and catatonic" for the last 2 days of his stay. He was then transferred to the emergency room of a third hospital for further medical evaluation. Upon admission (day 4 postingestion), he was found to be obtunded and unresponsive to verbal or painful stimuli. He had stable vital signs and was afebrile. His pupils were anisocoric (right pupil 5 mm, left pupil 2 mm) and sluggishly responsive to light. All four extremities were flaccid. The rest of his physical examination was unremarkable. He was intubated and placed on a ventilator while his workup continued.

A CT scan of the head showed hypodensities affecting the white matter diffusely, suggesting brain edema that was predominantly affecting the white matter. A lumbar puncture yielded clear cerebrospinal fluid (CSF) at a high opening pressure of 33 cm of H_2O (normal 8–20 cm of H_2O). Subsequent CSF studies revealed an elevated CSF protein of 64 mg/dL (normal 15–45 mg/dL). CSF cell counts were within normal range, antisyphilis antibodies were not detected (i.e., negative venereal disease research laboratory test), and bacterial cultures were negative for growth. Viral studies

[1]Office of the Chief Medical Examiner, 520 First Avenue, New York, NY 10016.
[2]CIIT Centers for Health Research, Six Davis Drive, Research Triangle Park, NC 27709.
[3]Division of Pulmonary, Critical Care and Sleep Medicine, Wayne State University, 3990 John R, Detroit, MI 48201.
[4]Department of Neurology, Wayne State University, 4201 St. Antoine, Detroit, MI 48201.
[5]Wayne County Medical Examiner Office, 1300 East Warren Avenue, Detroit, MI 48207.

Received 21 Jan. 2006; and in revised form 15 April 2006; accepted 23 April 2006; published 31 Aug. 2006.

on the CSF were negative for herpes simplex virus 1 and 2 and West Nile virus. During the next 2 days, he developed a distended bladder with overflow incontinence and decreased rectal tone. On the third day of hospitalization (day 7 postingestion) he was declared brain dead. On that day, soon after the family was notified of the results of the brain death protocol, he was found in asystole. Resuscitation was not attempted.

At autopsy, he was 5 ft, 8 in. tall (173 cm) and weighed 114 pounds (51.7 kg). He had mild flattening of the gyri and narrowing of the sulci, mild softening of the brain (early changes of "respirator brain"), and left lower lung lobe consolidation. Microscopic examination of the brain showed ischemic neuronal changes in the cortex and cerebellum. The white matter of the cerebrum, cerebellum, and pons was mildly to moderately spongy and vacuolated (Fig. 1*A*). The meninges and cerebrovasculature were free of significant inflammation, glial nodules were not observed, and no abnormal cellular inclusions were identified. Acute bronchoalveolar pneumonia was confirmed in the left lung. Postmortem toxicology studies were performed and consisted of screening for volatile solvents (alcohols), cyanide, salicylates, barbiturates, basic drugs, stimulants, benzodiazepines, cocaine groups, phencyclidine, opiates, and acetaminophen. These tests were positive only for low levels of lidocaine and promethazine, which were administered to him in the hospital.

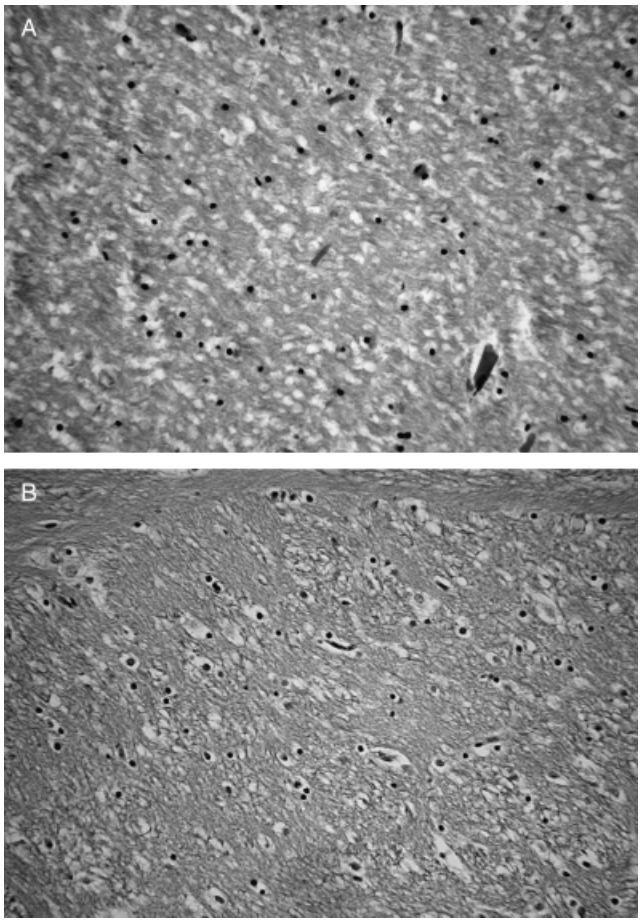

FIG. 1—*(A) Mild cerebellar white matter vacuolization in the deceased patient with suspected bromethalin neurotoxicosis (hematoxylin and eosin, photographed at 200 ×). (B) Similar lesions occur in cats following bromethalin ingestion (hematoxylin and eosin, photographed at 200 ×). Brain sample is from an experimental study that characterized the neurotoxicity of bromethalin in cats (5).*

Method

Chemical analysis for the presence of bromethalin, or its metabolite(s), in formalin-fixed liver and brain tissue samples from the deceased were performed at the CIIT Centers for Health Research. In addition, the following samples were also analyzed: (1) a bromethalin-based rodenticide (Real-Kill® Mouse Killer, United Industries Corporation, St. Louis, MO) similar to the ingested product; (2) formalin-treated rodenticide (1 g of Real-Kill® Mouse Killer which was placed in 20 mL of 10% formalin for 48 h before analysis); and (3) brain tissue from a naïve rat (no exposure to chemical).

All samples were prepared using a modified Folch extraction method (2) with tissue homogenization performed using a Mixer Mill MM301 ball mill (Retsch, Newtown, PA). Samples of the brain and liver were excised and diced into small pieces. Tissue and bait samples were weighed (~ 200 mg each) and placed into separate 5 mL conical bottom screw cap plastic vials along with 1.5 mL of deionized water, 1.5 mL of methanol, and two 3 mm stainless steel bearings. Homogenization was performed with the MM301 set to 30 Hz for 10 min. A 2 mL volume of chloroform was added to the samples, each of which was briefly mixed by hand before centrifugation at 4°C and 1500 × *g* (5 min). The top (chloroform) layer was removed and concentrated to *c*. 250 µL under a stream of nitrogen. The sample was transferred to a gas chromatography (GC) vial and analyzed by GC with mass spectrometric detection (GC/MS).

The GC/MS analysis was conducted using an Agilent 6890 gas chromatogram with a 5973 inert mass spectrometer. Cyclo double gooseneck injection port liners, 2 mm ID, and an Agilent DB-5MS capillary column (30 m × 0.25 mm ID) were equipped in the GC. Analysis of the sample extracts was conducted with the mass spectrometer in negative chemical ionization mode (NCI) and selective ion monitoring (SIM) of the following ions: 452, 467, 469, 498, and 543 atomic mass units (amu). These ions correspond to compounds containing two or three bromine atoms (based on isotope distribution patterns), which were observed in the formalin-treated rat bait. The following instrumental parameters were used with the GC/MS: injection port temperature was 250°C, column flow was set to 1.2 mL/min (splitless), and initial GC temperature was 150°C. The GC temperature program was as follows: initial temperature was held for 2 min, followed by a 20°C/min ramp to 300°C, which was held for 5.5 min. The MS transfer line heater was set to 250°C, the MS source was set to 150°C, and the MS quad was set to 150°C.

Results

The results of the analyses are illustrated in Fig. 2. A demethylated form of bromethalin (Fig. 3) was detected in the liver and brain. On the basis of the patient's initial self-report of ingestion of the poison, the clinical symptoms consistent with bromethalin poisoning, the supportive finding of white matter vacuolation at autopsy, the lack of other pathologic findings to otherwise explain the death, and analytical chemical confirmation of the presence of a demethylated form of bromethalin in the liver and brain, the cause of death was determined to be bromethalin poisoning. The manner of death was accident.

Discussion

No Pest® Mouse Killer Place Packs are small paper packages containing green, odorless pellets that are placed strategically to allow access to rodents. The active ingredient is [^{14}C]-bromethalin,

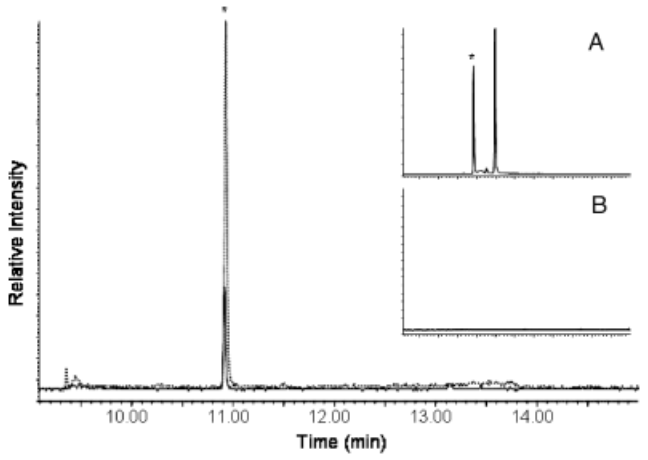

FIG. 2—*Gas chromatography with mass spectrometric detection-selective ion monitoring chromatogram (ion 543) demonstrating the presence of a demethylated form of bromethalin in the liver (solid line) and brain (dotted line) of the deceased. Inset A shows chromatogram from a formalin-treated bromethalin-based rodenticide (positive control). Incubation of the rat bait in formalin produced a number of bromethalin degradation products, including one with a mass spectra consistent with desmethylbromethalin (asterisk). The large peak to the right of desmethylbromethalin is bromethalin. Analysis of rat bait not exposed to formalin produced a single bromethalin peak (not shown). The chromatogram shown in inset B is from a formalin-fixed brain sample from a naïve rat (negative control).*

a potent neurotoxin. The presumed mechanism of action of bromethalin is uncoupling of oxidative phosphorylation resulting in depletion of cellular adenosine triphosphate (ATP) (3). Decreased ATP production disrupts the function of the sodium-potassium ion channel pumps leading to cerebral edema and elevated CSF pressure (3).

In animals, bromethalin is absorbed rapidly by the gastrointestinal tract (absorption half-life of 2.7 ± 0.2 h), and blood concentrations peak within *c*. 4 h (3). Biliary excretion in rats accounts for 5–25% of an orally administered dose of 0.5–1 mg bromethalin/kg (4). Urinary elimination of bromethalin is minimal and only accounts for 2.2% of an orally administered dose (4). A plasma half-life of 5.6 days has been reported in rats (4). A major route of hepatic bromethalin metabolism is through N-demethylation to desmethylbromethalin, which is *c*. 10- to 1000-fold more potent than bromethalin in inducing uncoupling of oxidative phosphorylation (3).

The toxicity of bromethalin varies among animals. Cats are highly sensitive, whereas guinea pigs are much more resistant (3,5). The LD_{50} of bromethalin (when administered in a rodenticide bait form) for cats was estimated to be 0.54 mg/kg with 95% upper and lower estimates of 0.40 and 0.71 mg bromethalin/kg, respectively (5). Dorman and coworkers (5) reported that the lowest observed lethal dose in a cat was 0.45 mg/kg. The sensitivity of humans to bromethalin has not been previously studied, and the LD_{50} in people is unknown. The deceased self-reported that he consumed eight packages of the rodenticide. An investigation confirmed that each package of rodenticide contains 0.75 ounce (21.2 g) of bait; therefore, the deceased ingested *c*. 17 mg of bromethalin (equivalent to a lethal dose of 0.33 mg/kg).

In cats, ingestion of doses less than the LD_{50} was associated with a delay in the onset of clinical signs for up to several days. Clinical signs seen in bromethalin-poisoned cats include progressive central nervous system depression, ataxia, paresis, and paralysis (5,6). Other clinical signs observed in poisoned dogs and cats include decreased conscious proprioception, focal motor seizures, decerebrate posturing (opisthotonus and limb rigidity), anisocoria, behavioral changes, semicoma, abdominal distension, and apparent urinary bladder paralysis (5,7). Ingestion of doses greater than the LD_{50} in dogs and rats caused an acute convulsant syndrome (onset of less than 16 h) characterized by hyperthermia, muscle tremors, hyperexcitability, and generalized seizures with death occurring in as little as 8 h (3,7). This acute syndrome was not observed in cats (5).

Nonspecific electroencephalographic (EEG) abnormalities have been shown in dogs and consisted of marked voltage depression, spike and spike-and-wave patterns, abnormal high-voltage slow wave activity, and photoconvulsive or photoparoxysmal responses (8). Similar EEG changes including voltage depression, crude spikes, and high voltage slow wave and spike abnormalities were also seen in bromethalin-poisoned cats (9). The presence of these EEG findings may support the diagnosis of bromethalin poisoning in either animals or humans. In this case, an EEG performed on the 6th day of the clinical course (the day before death) showed diffuse slowing with low amplitude voltage, which has been seen in bromethalin-exposed animals but is also a nonspecific finding consistent with global ischemia and impending brain death.

The predominant light microscopic finding in animals treated with bromethalin consists of varying degrees of vacuolization of the white matter of the central nervous system with no corresponding inflammation or cell death (Fig. 1*B*). Mild microgliosis, mild optic nerve vacuolization, and occasional megakaryocytes in the spleen have also been reported (10,11). Electron microcopy of vacuolated white matter has shown intramyelinic edema with splitting of the myelin at the interperiod line (3,10).

The differential diagnosis for the clinical presentation varies considerably and includes injury and encephalitis. The pathologic white matter vacuolation is comparable to that seen in carbon tetrachloride and hexachlorophene poisoning (12,13). No diagnostic antemortem test to confirm bromethalin exposure has been established to date, and no known prior attempt has been made to analyze formalin-fixed tissue. The diagnosis is made based on a history of exposure to a potentially toxic dose, clinical signs, histologic presence of white matter vacuolization, and detection of bromethalin in tissue, when possible (10).

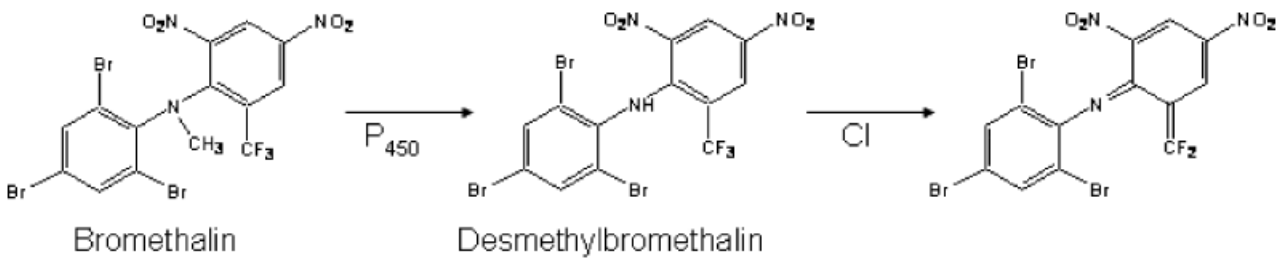

FIG. 3—*N-demethylation of bromethalin (577 amu) by hepatic microsomal enzymes results in the formation of desmethylbromethalin (563 amu). Desmethylbromethalin undergoes additional rearrangements (loss of HF, 543 amu) during ionization by the mass spectrometer.*

Chemical confirmation of bromethalin poisoning can prove challenging. Chemical analysis of necropsy specimens taken from domestic animals poisoned with bromethalin has demonstrated measurable pesticide residues in fat, liver, kidney, and brain tissues (10,14). Bromethalin readily undergoes photodegradation (10); thus, samples intended for chemical analysis should be protected from the light. To our knowledge this case report is the first to attempt analysis of formalin-fixed tissue to confirm a diagnosis of bromethalin poisoning. Our experiment with formalin-treated rodenticide pellets confirmed the chemical stability of bromethalin in this media. GC/MS analysis of formalin-fixed liver and brain samples from the deceased confirmed the presence of a demethylated form of bromethalin in the patient's liver and brain. Because of the lack of an analytical standard, no attempt was made to quantitate this analyte.

Parallel findings between the clinical course of animal models and this human poisoning include the delay in clinical symptoms/signs, behavioral changes, anisocoria, cerebral edema, elevated CSF pressure, and subsequent death. The postmortem finding of white matter vacuolization in the deceased mimicked lesions seen in animals and further supports the diagnosis of bromethalin poisoning. In this case, the ischemic neuronal changes seen on microscopic examination are likely related to his terminal diagnosis of brain death and do not necessarily reflect bromethalin toxicity.

Treatment of bromethalin poisoning in the first 4 h of cats exposed to less than 0.1 mg/kg consists of induction of emesis and administration of activated charcoal to prevent gastrointestinal absorption. Subsequent repeated doses of activated charcoal are recommended for doses over 0.1 mg/kg (15). The increased efficacy of repeated activated charcoal administration is likely due to its ability to disrupt the enterohepatic recirculation of bromethalin (8). Unfortunately, even repeated administration of activated charcoal may not be very effective, because delayed deaths were observed in cats 15–19 days after bromethalin administration despite the initial administration of four doses of activated charcoal (5). After the absorptive phase, continuous dexamethasone, and mannitol therapy have been associated with clinical improvement in animals that were sublethally dosed with bromethalin (3). Several experimental studies have examined whether the combined administration of mannitol and dexamethasone could be effective in managing dogs or cats given a lethal dose of bromethalin (5,16). Unfortunately, these therapies were neither effective in preventing signs from developing nor in reversing the neurotoxic syndrome once it had developed. Dogs and cats with significant neurologic signs consistently have a grave prognosis despite treatment aimed at reducing cerebral edema (8).

In this case, it appears that an undiagnosed and untreated psychiatric disorder led to the consumption of the poison. According to the family of the deceased, he had been acting increasingly strange over the last 2 years of his life with a notable escalation in bizarre behavior the week before his ingestion of the poison. In the 2 years before his death, he was often found staring blankly, he complained that he was "seeing dead people," and he admitted to his mother that he had auditory hallucinations. Although his family strongly denied that he had any history of drug abuse, it is possible that illicit drug abuse could have contributed to his abnormal thoughts and behavior; the initial positive urine barbiturate screen was never accounted for in the medical records reviewed. However, the deceased was at an age when the symptoms of schizophrenia most commonly manifest, and it is likely that he would have been diagnosed with this illness or a similar psychiatric disorder if he had lived.

Because the death occurred after the intentional consumption of poison, it would be reasonable to alternatively classify the manner of death as a suicide. However, the deceased never indicated a wish to kill himself. His clear intention was to "kill the rats inside." Furthermore, in his altered mental state at the time that he ate the rat bait, it is unlikely that he fully comprehended the potential lethality of his actions upon himself. Therefore, his death was ruled an accident, and an acute psychotic episode was listed on the death certificate as a significant contributing condition.

Adults and children have historically ingested rat poison accidentally or with suicidal or homicidal intentions. An increased awareness of the availability of bromethalin-based rodenticides and the clinical and pathologic findings of toxicity will improve recognition of human poisonings. We anticipate that the management of exposed humans will use therapeutic strategies developed for animals, i.e., gastrointestinal decontamination, control of cerebral edema, and other supportive measures.

References

1. Watson WA, Litovitz TL, Rodgers GC Jr, Klein-Schwartz W, Reid N, Youniss J, et al. 2004 Annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. Am J Emerg Med 2005;23(5):589–666.
2. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497–509.
3. Van Lier RBL, Cherry LD. The toxicity and mechanism of action of bromethalin: a new single-feeding rodenticide. Fundam Appl Toxicol 1988;11:664–72.
4. VanLier RBL, Ottosen LD. Studies on the mechanism of toxicity of bromethalin, a new rodenticide. Toxicologist 1981;1:114–5 [Abstract].
5. Dorman DC, Parker AJ, Dye JA, Buck WB. Bromethalin neurotoxicosis in the cat. Prog Vet Neurol 1990;1:189–96.
6. Martin T, Johnson B. A suspected case of bromethalin toxicity in a domestic cat. Vet Hum Toxicol 1989;3:239–40.
7. Dorman DC, Parker AJ, Buck WB. Bromethalin toxicosis in the dog I: clinical effects. J Am Anim Hosp Assoc 1990;26:589–94.
8. Dorman DC, Parker AJ, Buck WB. Electroencephalographic changes associated with bromethalin toxicosis in the dog. Vet Hum Toxicol 1991;33(1):9–11.
9. Dorman DC, Parker AJ, Schaeffer DJ, Buck WB. Quantitative and qualitative electroencephalographic changes in normal and bromethalin-dosed cats. Prog Vet Neurol 1990;1(4):451–9.
10. Dorman DC, Harlin KS, Simon J, Buck WB. Diagnosis of bromethalin poisoning in the dog. J Vet Diag Invest 1990;2:123–8.
11. Dorman DC, Zachary JF, Buck WB. Neuropathologic findings of bromethalin toxicosis in the cat. Vet Pathol 1992;29:139–44.
12. Ellison D, Love S, editors. Neuropathology. London: Mosby International Ltd., 1998.
13. Martinez AJ, Boehm R, Hadfield MG. Acute hexachlorophene encephalopathy: clinico-neuropathological correlation. Acta Neuropathol (Berl) 1974;28:93–103.
14. Braselton WE, Johnson M. Thin layer chromatography convulsant screen extended by gas chromatography-mass spectrometry. J Vet Diagn Invest 2003;15:42–5.
15. Dunayer E. Bromethalin: the other rodenticide. Vet Med 2003;732–6.
16. Dorman DC, Parker AJ, Buck WB. Bromethalin toxicosis in the dog II: treatment of the toxic syndrome. J Am Assoc Anim Hosp 1990;26:595–8.

Additional information and reprint requests:
Melissa A. Pasquale-Styles, M.D.
Office of the Chief Medical Examiner
520 First Avenue
New York, NY 10016
E-mail: pasqualemd@hotmail.com