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Tissue Residue Levels of the Tranquilizer Combination of Butorphanol, Azaperone, and Medetomidine, and the Antagonists, Naltrexone, Atipamezole, and Tolazoline, in Black Bears (*Ursus americanus*) Postimmobilization

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ABSTRACT: The tranquilizer combination of butorphanol, azaperone, and medetomidine (BAM) has shown good efficacy for immobilization of wildlife, including black bears (*Ursus americanus*). BAM is antagonized with a combination of naltrexone and atipamezole. We immobilized 19 adult captive wild caught black bears and, except for three bears that were euthanized immediately, bears were recovered with naltrexone and atipamezole. Tissue residues (≥0.01 ppm) for the tranquilizers butorphanol, azaperone, and medetomidine were detected in liver and muscle of all three bears euthanized on day 0 postinjection (PI). Azaperone was not detected after 1 d PI. Residue for medetomidine was detected in two bears: in the liver 3 d PI and in the kidney 6 d PI. Butorphanol was reported in three bears: in fat 5 d PI, in kidney 6 d PI, and, surprisingly, in kidney, muscle, and fat 7 d PI. No tissue residues were detected in the three bears euthanized at 8 d PI. Tissue residues for the antagonists, naltrexone and atipamezole, were detected in bears euthanized 2 and 6 d PI, but not in tissues from animals euthanized at 7 or 8 d PI.

Key words: Atipamezole, azaperone, black bear, butorphanol, medetomidine, naltrexone, tissue residue, *Ursus americanus*.

There are very few drugs approved by the US Food and Drug Administration (FDA) that are labeled for use in wildlife, and therefore, most usage is considered extra label and falls under the US Animal Medicinal Drug Use Clarification Act (AMDUCA; title 21, Code of Federal Regulations, part 530). In addition, most of these animals are hunted and therefore subject to FDA rules and policies intended to ensure that food derived from animals treated with drugs is safe for human consumption. Under AMDUCA, a veterinary practitioner should "take appropriate measures to assure that assigned time frames for withdrawal are met and no illegal drug residues occur in any food producing animal subjected to extra-label treatment" (Papich 1996; US FDA 2019). The withdrawal period allows time for a drug (or its component parts) in edible tissues of a treated animal to reach concentrations below a regulatory threshold of tolerance, although technically no amount of residue is tolerated for drugs used off-label.

The tranquilizer combinations of butorphanol, azaperone, and medetomidine (BAM; Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) and similar combination of nalbuphine, azaperone, and medetomidine (NalMed-A; Wildlife Pharmaceuticals) have shown good efficacy for immobilization of wildlife, including black bears (*Ursus americanus*; Wolfe et al. 2008, 2014). The few published tissue drug residue studies for anesthetics in wildlife include BAM in white-tailed deer (*Odocoileus virginianus*; Cook et al. 2016), NalMed-A in wapiti (*Cervus canadensis*; Wolfe et al. 2018), and tiletamine/zolazepam in polar bears (*Ursus maritimus*; Semple et al. 2000). Although no withdrawal has been established in food animals for opioids, Papich (1996) indicated that a reasonable estimate would be 48 hr. Butorphanol, a narcotic agonist-antagonist analgesic, is metabolized by the liver and excreted through urine and bile (Plumb 1999). Although no withdrawal period for butorphanol has been established in food animals, the serum half-life in dogs was reported as less than 2 hr and the duration of action was twice that (Padrid and Church

2008). Azaperone, a butyrophenone agonist, is metabolized by the liver, excreted in the feces, and has a 3-d withdrawal time for pigs in the UK (Rauws and Olling 1978; Papich 1996). Medetomidine is a potent alpha 2-adrenoreceptor agonist which is metabolized by the liver and is excreted in the urine and feces with reported serum half-lives ranging from 0.97 to 1.60 hr (Salonen 1989). The antagonists for BAM and NalMed-A—naltrexone and atipamezole—are both metabolized in the liver and excreted in the urine (Plumb 1999). Tissue residues for BAM components and antagonists were not detected in white-tailed deer at 11 or 21 d after treatment (Cook et al. 2016). In elk immobilized with NalMed-A and antagonized, tissue residues were not detected ≥6 d postimmobilization (Wolfe et al. 2018).

To assess drug persistence in tissues as a basis for withdrawal recommendations, we used black bears already captured and slated for euthanasia because of management conflicts per Colorado Division of Parks and Wildlife Administrative Directive. Our procedures were reviewed and approved by the Colorado Division of Parks and Wildlife Animal Care and Use Committee (file 05-2017). Bears were held at the Foothills Wildlife Research Facility in covered outdoor enclosures (6×12 m heavy chain link) with access to a den box, hide, and natural cover. They were fed a variety of fruits, peanuts, bakery goods, and vegetables for enrichment and either dog kibble or Wild Carnivore Bear Maintenance diet (Mazuri Exotic Animal Nutrition, St. Louis, Missouri, USA), ad libitum.

Bears were immobilized with a combination of 27.3 mg/mL butorphanol, 9.1 mg/mL azaperone, and 10.9 mg/mL medetomidine by intramuscular injection by a district wildlife manager handling the incident and then transported to Foothills Wildlife Research Facility or brought in awake in the trap and immobilized and transferred to the holding cage. The dose was 1 mL BAM per 45 kg estimated mass for an estimated dosage of 0.6 mg/kg butorphanol, 0.2 mg/kg azaperone, and 0.2 mg/kg medetomidine. Except for day 0, bears were given antagonists either when placed in the transport cage or when transferred to the holding cage, depending on the capture circumstances. The dose for atipamezole was 2 cm^3 per 1 cm^3 BAM for an estimated dosage of 1.1 mg/kg or five times the dose of medetomidine. The dosage for naltrexone was 50 mg per bear. One bear on the day 6 assessment did not receive naltrexone.

Bears were euthanized by captive bolt at the day 0 sample time while still under BAM sedation and at ≥2 d postimmobilization after dart or pole syringe injection with tiletamine/zolazepam (1–2 mg/kg; Telazol®, Zoetis, Parsippany, New Jersey, USA). If any of the drugs were detected in a bear at a time point, we then moved to the next time point. Consequently, at some time points only one or two bears were tested. We terminated the study when all three bears tested negative for detectable tissue residues for all target drugs. Liver, kidney, fat, and skeletal muscle were collected and frozen at −70 C until submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory (Texas A&M University, College Station, Texas, USA) for drug residue analysis. Tissue residues were analyzed by liquid chromatography-tandem mass spectrometry after isolation by Solid Phase Extraction. The limit of detection for the assay is 0.01 ppm. Results are not quantified, and consequently positive tests indicated ≥0.01 ppm of the drug in that tissue sample. Tissue residue tolerance limits for these drugs have not been established in the US.

Tissue residues (≥0.01 ppm) for the tranquilizers butorphanol, azaperone, and medetomidine were detected in liver and muscle of all three bears euthanized on day 0 postinjection (PI; Table 1). Azaperone was not detected after 1 d PI. Residue for medetomidine was detected in a few tissues of one bear: in the liver 3 d PI and in the kidney 6 d PI. After its absence in samples from bears euthanized 3 and 4 d PI, butorphanol was reported in fat 5 d PI, in kidney 6 d PI, and most surprisingly, in kidney, muscle, and fat in two bears on day 7 PI (Table 1). One bear liver also initially

TABLE 1. Drug tissue residues detected in black bears (*Ursus americanus*) sedated with butorphanol, azaperone, and medetomidine; antagonized with atipamezole and naltrexone; and then euthanized and sampled at intervals over an 8-d period after immobilization and recovery (where allowed). Liver, kidney, fat, and skeletal muscle tissue samples from each subject were screened. "All" means the respective drug was detected in all four tissues at that time point; otherwise, only the specific tissues listed parenthetically had detectable drug.

		No. black bears with drug residues (tissues)				
Days PI[a]	*n*	Butorphanol	Medetomidine	Azaperone	Atipamezole	Naltrexone
0	3	3 (all)	3 (all)	3 (all)	NA[b]	NA
2	1	trace (all)	0	0	1 (liver)	0
3	2	0	1 (liver)	0	0	0
4	3	0	0	0	1 (liver, fat)	0
5	1	1 (fat)	0	0	0	0
6	3	2 (kidney)	1 (kidney)	0	1 (kidney)	NA
7	3	2 (kidney, fat) 1 (muscle)	0	0	0	0
8	3	0	0	0	0	0

[a] Days PI = days euthanized posttreatment with butorphanol, azaperone, and medetomidine.
[b] NA = not applicable.

tested positive for butorphanol on day 8 PI, but because butorphanol had not been detected in any of the liver tissue samples prior to that time period we resubmitted these tissues and no tissue residues were detected in the three bears euthanized at 8 d PI (Table 1). No archived tissues were available for retesting bears sampled 5, 6, and 7 d PI, so we have reported the original results although we remain skeptical that butorphanol was present based on the overall residue pattern and apparent problems with assay specificity during the time period when these samples were submitted for testing. Tissue residues for the antagonists, naltrexone and atipamezole, were detected in the bears euthanized 2 and 6 d PI, but not in tissues from animals euthanized at 7 and 8 d PI (Table 1). As expected, tiletamine and zolazepam were detected in one or more tissues from all bears immobilized with this drug combination immediately prior to euthanasia at all time points. The apparent absence of tissue residues for BAM components after 7 d or of antagonists after 6 d should be useful in developing drug withdrawal guidelines for using this immobilization combination in black bears.

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