Euthanasia is the humane termination of an animal’s life. According to the AVMA Guidelines for the Euthanasia of Animals, methods of euthanasia must cause rapid loss of consciousness followed by cardiac or respiratory arrest and death and must minimize pain, distress, and anxiety prior to loss of consciousness. Evaluation of euthanasia methods for birds is limited, and the available peer-reviewed reports primarily address euthanasia and slaughter methods for commercially raised poultry. In ornithological research, birds are collected in the field for specific purposes such as procurement of tissue or for use as specimens in museums and teaching collections. Euthanasia of wild birds may also be necessary to relieve pain or address welfare concerns when a bird is injured or debilitated during collection such that it has a low probability of survival. The AVMA recognizes that recommended modes of euthanasia for captive animals are not always feasible in field situations. However, the challenges presented by field conditions do not release investigators from the responsibility of minimizing the pain and distress of animals to be euthanized.
The AVMA euthanasia guidelines include methods that are acceptable or acceptable with conditions for euthanasia of birds. The IV injection of a euthanasia agent, such as pentobarbital, is currently indicated as an acceptable method. The guidelines state that this is the quickest and most reliable method when it can be performed without causing fear or distress; however, wild, fearful, or excited birds might require a sedative or anesthetic before IV injection can be performed.\(^1\) It is generally understood that the capture and handling of unsedated wild birds induce fear and distress. In addition, obtaining venous access in a small bird requires training and skill and often increases handling time.\(^2\) Therefore, although IV injection of pentobarbital is considered a humane ending, minimizing fear and distress associated with handling is still a concern.

The most common AVMA-approved method preferred by veterinarians for euthanasia of companion birds is to anesthetize or sedate a bird prior to IV administration of an overdose of pentobarbital.\(^3,4\) Pentobarbital sodium is labeled for use in the United States and Canada for euthanasia of all animal species by IV administration, although it is not FDA-approved for use in birds. As a controlled substance (US Department of Justice–DEA schedule II drug), it can be used only by or under the supervision of a licensed veterinarian. Furthermore, data supporting the effects of IV administration of pentobarbital to birds other than poultry, including times to unconsciousness or death, are not available.

Wildlife biologists, wildlife managers, and wildlife health professionals are often required to procure specimens from wild avian populations using capture methods such as mist nets or net guns. For remote collecting or international fieldwork by field biologists and those without a license to administer anesthetics and controlled drugs, the administration of injectable drugs as a means to perform or aid euthanasia of birds is often illegal, and the use of inhalation anesthetics in remote areas is often impractical; therefore, physical methods must be used to secure the specimen. Some physical methods, such as cervical dislocation and decapitation, can cause irreparable damage to the cadaver and are not suitable when collecting specimens for research and archiving in museums. Thoracic compression is the preferred method used by field biologists because it yields a quick death and high-quality specimens.\(^4\) Currently, the AVMA considers TC to be an unacceptable method of euthanasia for sentient birds because data supporting this method, including levels of pain, distress, and times to unconsciousness or death, are not available.\(^1\) To the authors’ knowledge, no such data are available for any method of euthanasia in nonpoultry avian species.

The use of EEG activity to determine when consciousness is lost is considered by some investigators to be the most objective means of assessing unconsciousness,\(^6,7\) while others believe that the EEG is not a direct measure of consciousness and does not determine the exact moment when unconsciousness occurs because changes can be gradual and subtle.\(^8\) However, there is sufficient agreement that an isoelectric EEG pattern (also known as a flatline pattern or electocerebral inactivity) is not compatible with consciousness and is an indicator of cessation of normal cerebral function.\(^9\) Electroencephalography results have been used in welfare evaluations of poultry slaughter and euthanasia techniques.\(^7,9,10\) Additionally, an isoelectric EEG pattern combined with nonreversible states such as apnea or cessation of pulse has been used to define death.\(^7,9,10\)

The objective of the study reported here was to compare an overdose of pentobarbital sodium solution (as IPT) to TC as methods of euthanasia for small passerine birds, with 2 common species used as subjects. The study was designed to compare time to circulatory arrest, time to isoelectric EEG, and other endpoints of interest between the 2 euthanasia methods among birds within each species, but it was not designed to compare outcomes between the 2 species. To our knowledge, no previous studies have evaluated changes in cerebral electrical activity and other physiologic effects of the 2 methods of euthanasia in anesthetized passerine birds. We hypothesized that there would be no difference in the time to cessation of arterial pulses or to isoelectric EEG between the 2 methods. Gross and histologic postmortem data were collected and reported to provide information necessary to understand the physical cause of death following TC.

**Materials and Methods**

**Birds and treatment allocation**

The study was conducted with wild-caught adult house sparrows (*Passer domesticus*) and European starlings (*Sturnus vulgaris*). These species were selected to represent 2 body sizes (and the typical size range involved in most avian field studies globally) and because both species are considered overpopulated and invasive in North America. The capture of wild sparrows and starlings was conducted by 2 experienced investigators (AE and IEE). Sparrows were captured by use of mist nets and were promptly removed from the nets and placed into small holding pens (30.8 × 30.8 × 61.6 cm; 2 birds/pen). Pens were covered with a blanket and shuttled to the study facility at 2-hour intervals until the desired number of birds was collected. Starlings were captured before sunrise by use of hand nets at a large roost (estimated as > 250,000 birds). Birds were quickly removed from the net and placed into a small holding pen (30.8 × 30.8 × 61.6 cm; 2 birds/pen), which was covered with a blanket for transfer to the study facility (arriving approx 30 minutes after capture). Methods for netting birds followed the Ornithological Council Guidelines to the Use of Wild Birds in Research,\(^3\) and the protocols were approved by the University of California-Davis Institutional Animal Care and Use Committee.
Because mist netting of nontarget species could not be excluded during sparrow capture, a federal banding permit (No. 23383, issued to AE) was in place. All migratory birds caught in nets were released immediately at the point of capture. The study was approved by the University of California-Davis Institutional Animal Care and Use Committee.

In the present study, euthanasia end points were measured and anesthetized birds were used. These included 12 sparrows (body weight, 25.7 ± 2.0 g) and 12 starlings (body weight, 71.1 ± 3.4 g). The sample size was selected with the welfare of research animals in mind; a pilot investigation with 10 Japanese quail (involved in another study for which euthanasia was required) was performed to refine the procedures (data not shown), and the number of birds was kept small to reduce the number of animals used. Birds of each species were arbitrarily assigned to the TC and IPT groups (6 birds/species/group). Sexes were confirmed at necropsy. For the sparrows, the TC group included 5 males and 1 female, and the IPT group included 6 males. For starlings, the TC group included 3 males and 3 females, and the IPT group included 4 males and 1 female; sex of 1 bird was undetermined.

Another 6 birds that had been captured were anesthetized and euthanized by TC or IPT at the conclusion of the study to be examined by necropsy. These included 5 sparrows (3 females and a male that had TC and 1 female that had IPT) and 1 female starling that had TC. All birds that were brought into captivity were euthanized during or at the conclusion of the study.

Housing and husbandry

Sparrows and starlings were collected and housed at separate sequential periods to minimize time in captivity. Birds were housed in groups of 3 or 4 (sparrows) or 2 (starlings) in 27.5 × 20.0 × 15.0-in wire mesh cages at ambient temperature (approx 21°C to 26°C) with a 12-hour light to dark cycle. Water was available ad libitum in a water bowl and by drip line in each cage. Sparrows were provided with a commercial seed mix, and starlings were provided with a mixture of dry dog food and poultry mash daily.

Anesthetic and monitoring procedures

Birds were anesthetized with isoflurane in oxygen delivered via a small mask covering the head for induction. Isoflurane (2% to 4%) in oxygen (2 L/min) was delivered to the mask through a nonrebreathing circuit. The birds were endotracheally intubated, and isoflurane and oxygen flow were reduced to 2% and 1 L/min, respectively. Sparrows were intubated with a 16-gauge IV catheter with a silicone tip, and starlings were intubated with a 2-mm uncuffed endotracheal tube. A mechanical ventilator was used during placement of monitoring equipment, but birds were allowed to breathe spontaneously when data collection began. A side port on the endotracheal tube was connected to a monitor for measurement of PETCO2, end-tidal isoflurane concentration, and FiO2. The FiO2 began at 1.0 for all birds and was lowered during the course of anesthesia, with a goal of reaching 0.21 (equivalent to field applications without supplemental oxygen administration) prior to euthanasia. Ten starlings and 9 sparrows had the FiO2 maintained at ≤0.40, including 6 birds at FiO2 of 0.21. Two starlings and 3 sparrows had the FiO2 maintained at ≥0.70.

A Doppler crystal with gel applied was placed over the right carotid artery to monitor pulse rate. An intravesophageal temperature probe was inserted to the level of the thoracic esophagus. The equipment used for EEG and ECG was a digital electrophysiological monitoring system with integrated video monitoring. Feathers were plucked from the skin over the region of the skull to facilitate electrode placement. Five 25-gauge braided subdermal wire electrodes were placed SC in contact with the calvarium in the following positions: rostral to the opening of the left (A1) and right (A2) auditory canals, on the dorsal midline between A1 and A2 (C2), between C2 and A1 (C1), and between C2 and A2 (C4; Figure 1). A ground electrode was inserted rostral to the elec-

Figure 1—Schematic drawings of electrode placement on the dorsal aspect (A) and lateral aspect (B) of the head of a bird for EEG recording in a study to compare aspects of euthanasia by TC and IPT in wild-caught house sparrows (Passer domesticus) and European starlings (Sturnus vulgaris). Electrodes (represented by gray circles) were placed SC in contact with the calvarium of anesthetized birds in the following positions: rostral to the opening of the left (A1) and right (A2) auditory canals, on the dorsal midline between A1 and A2 (C2), between C2 and A1 (C1), and between C2 and A2 (C4). A ground electrode was inserted rostral to C2 (Z).

AJVR • Vol 78 • No. 8 • August 2017 889
trode designated as C. Additional electrodes were placed on the proximal aspect of the left wing and on the left thigh for ECG monitoring. Data were displayed with a transverse bipolar montage (pairs A1-C3, C3-Cz, Cz-C4, and C4-A2, and ECG). The sampling rate was 500 Hz, with sensitivity set at 5 µV/mm (100 µV/mm for ECG), sweep speed of 10 s/screen, a time constant of 0.1 seconds, and high-frequency filter of 70 Hz. To reduce electrical interference, a 60-Hz notch filter was applied.

In all birds, the medullary space of a tibiotarsal bone was catheterized with a 25-gauge, 5/8-in needle to administer propofol. Intraosseous catheter placement was preferred over an IV catheter for its ease of placement, minimal blood loss, and repeatability in the small passerines. Following instrumentation, isoflurane administration was discontinued, and a continuous rate infusion of propofol (via the intraosseous needle) was used to maintain anesthesia. The mean ± SD last end-tidal isoflurane concentration prior to initiation of propofol administration was 0.54 ± 0.26% for sparrows and 0.43 ± 0.09% for starlings. The propofol dose to obtain and maintain a light plane of anesthesia ranged from 5.7 to 13.2 mg/kg/min for sparrows and from 1.9 to 5.6 mg/kg/min for starlings. A light plane of anesthesia in propofol-anesthetized birds was identified by physical signs and an EEG showing continuous activity devoid of burst suppression (an EEG pattern characterized by a mixture of high-voltage electrical activity with periods of no activity; Figure 2). The duration of time that birds were without inhalant anesthesia and titrated with propofol to eliminate burst suppression varied.

**Euthanasia procedures**

For pentobarbital sodium administration, propofol infusion was stopped, and pentobarbital solution (392 mg/mL) was slowly injected (duration < 60 seconds) through the intraosseous catheter. A fixed volume of 0.05 mL of pentobarbital was delivered to sparrows, resulting in a dose range of 693 to 754 mg/kg, twice the recommended dose of 0.2 to 1 mL/kg. The first 2 starlings that underwent the procedure received 100 mg of pentobarbital/kg; however, the time to cessation of arterial pulse as measured by the Doppler method was prolonged (> 5 minutes) in 1 of these birds, so the remaining 4 starlings received a pentobarbital dose of 220 mg/kg; both doses were within the recommended range. With the exception of the prolonged time to cessation of pulse in 1 bird, all of the monitoring parameters and time intervals for these 2 birds were within the range obtained for the starlings that received the 220-mg/kg dose. Therefore, data from these birds were included in the analysis.

For the TC technique, propofol administration was stopped and TC was performed by 2 of the authors who were trained and highly experienced in this method (AE and IE). Field application of TC involves holding the bird to ensure proper finger placement. In all cases, the bird should be handled with both hands, and the dominant hand should be used for the underwing positions and compression, with the nondominant hand used for keel positioning and

![Figure 2](image-url)—Representative EEG and ECG recordings from a starling anesthetized with isoflurane (A) and a continuous rate infusion of propofol (by intraosseous administration; B). Notice the burst suppression pattern (a mixture of high-voltage electrical activity with periods of no activity) associated with isoflurane anesthesia. The ECG tracings were obtained from electrodes placed on the proximal aspect of the left wing and proximal part of the left pelvic limb. The calibration voltage applies to EEG only. See Figure 1 for remainder of key.
stability. With the bird’s keel against the palm of the nondominant hand, the bird is held with the thumb and index or middle finger of the dominant hand (approaching from the dorsum) under the bird’s wings. The thumb and finger are positioned on either side of the body cavity so that they are opposite each other in the triangular region formed by the pectoral muscle (ventrally), coracoid (cranially), and scapula (dorsally; Figure 3). The bird is readjusted in the nondominant hand so that the forefinger and middle finger are placed against the ventral edge of the keel, just below the furculum, to keep the bird in proper position when TC is initiated.13 The thumb and index or middle finger are then pinched rapidly together to stop cardiac activity and held in place for ≥ 60 seconds. Because the study birds were anesthetized and attached to monitoring equipment, the described field method was modified as follows: the bird was placed in ventral recumbency with the sternum on the examination table. The researcher placed the dominant hand over the wings, gently lifting them to position the index finger and thumb in the correct placement. The table surface was used to keep the sternum stable, replacing the nondominant hand. The result was similar to the field method, with the table surface providing stability while TC was applied with the dominant hand. The result was similar to the field method, with the table surface providing stability while TC was applied with the dominant hand. Monitoring was stabilized before TC was applied, and then the forefinger and thumb were rapidly and firmly pressed together to compress the heart. The bird was not lifted from the table during this time. The pinch was maintained for ≥ 60 seconds for each bird.

Necropsy procedures

Postmortem radiographs (ventrodorsal and right lateral projections) of sparrows (n = 6) and starlings (6) euthanized by TC were obtained. Digital radiography was performed with settings of 55 to 60 kVp and 5 mAs. The birds were placed directly against the detector panel without use of a radiographic grid. Carcasses were stored at 4°C within 4 hours after death, and gross examination or tissue fixation was performed within 48 hours after death. Most starlings (n = 6 and 5 that were euthanized by TC and IPT, respectively) were examined as fresh carcasses. Skin was removed from the ventral aspect of the body,

Figure 3—Schematic drawing of anatomic points and finger placement for TC overlaid on a digital photograph of the left body wall of a starling. The thumb and index or middle finger of the dominant hand are placed on opposite sides of the thorax in a space ventral to the scapula, dorsal to the coracoid, and caudal to the humerus when the humerus is extended above the body (yellow circle); the dorsum of the bird is against the palm of this hand during the procedure. With correct finger placement, cardiac contractions are easily palpable. In field applications, the fore and middle fingers of the opposite hand are placed along the ventral aspect of the sternum for additional support (blue circle); in the study, anesthetized and instrumented birds were placed in ventral recumbency on the flat surface of an examination table, and finger placement on the sternum was not used. Rapid, firm compression (maintained for ≥ 60 seconds) was performed with the thumb and forefingers over the heart.

Figure 4—Box plots of times from initiation of euthanasia to end points of interest (time to cessation of arterial pulse as detected by Doppler method [Pulse], apnea as determined by capnography and observation of video recordings [Resp], and isoelectric EEG signal [EEG]) in anesthetized wild-caught house sparrows (n = 6; A) and European starlings (6; B) euthanized by TC or IPT. The horizontal line within each box represents the median, and boxes represent the range from the first to third quartiles. Whiskers represent the minimum and maximum values within 1.5 times the interquartile range of the median, and white circles represent values outside that range. The horizontal dashed line in each panel indicates the maximum time that the TC pinch was held. Asterisks indicate significant (P < 0.05) differences between IPT and TC groups. For the sparrows in the TC group (A), notice that cessation of pulses was immediate for all but 1 animal, so the median, box, and whiskers were all superimposed at the x-axis.
and the body wall, including the ribs and clavicles, was cut along the costal margins to remove the keel and the attached pectoral musculature. The heart and viscera were examined in situ, and any coelomic hemorrhage was noted. The trachea, esophagus, and associated soft tissues were cut away from the neck, and the viscera (excluding the lungs) were removed from the body. The heart and great vessels were dissected free from surrounding tissues, and any pericardial or perihemorrhage and gross tissue damage were noted. The lungs were removed and examined separately, and any gross tissue damage was noted.

Tissues from all sparrows (n = 10 and 7 euthanized by TC and IPT, respectively) and 2 starlings (1 euthanized by each method) were fixed in neutral-buffered 10% formalin solution prior to dissection. The keel was removed from the starlings and most of the sparrows (n = 12) as previously described, the presence of coelomic hemorrhage was recorded, and the head, limbs, and skin were removed before fixation of tissues for ≥3 days. After fixation, dissection proceeded as previously described.

Five sparrows (4 and 1 that underwent TC and IPT, respectively) were skinned and then fixed with the keel intact after removal of the head and limbs. After fixation, the keel was removed as described above and the viscera (including the lungs) were dissected free. Gross examination was performed by use of a dissecting microscope, and specimens were then prepared for histologic examination. The viscera were then trimmed en bloc and sectioned in the sagittal plane to include the heart, lungs, cranial viscera were then trimmed en bloc and sectioned at a thickness of 4 µm, and stained with H&E stain. Step sections were embedded in paraffin, sectioned at a thickness of 4 µm, and stained with H&E stain. Step sections were made as necessary to evaluate structures of interest. Histologic sections were evaluated by a board-certified veterinary pathologist (MKK) who was blinded to the methods of euthanasia for these 5 birds.

**Data analysis**

Continuous recordings of monitoring data were captured during the procedure. End points were defined as apnea determined by capnography (to determine that ventilation ceased) and by observation of coelomic movement or excursions on video recordings; cessation of pulse as detected by the Doppler crystal; onset of any abnormal ECG pattern (ie, arrhythmia, fibrillation, loss of ventricular complexes, or asystole); and onset of isoelectric EEG as measured from recordings by use of the end of detectable electrical activity determined not to be artificial. Time from the onset of the euthanasia technique (beginning of the pentobarbital injection [IPT] or application of the compressive pinch [TC]) to each of the end points, as well as the time between the different end points, was measured for each bird by means of a stopwatch and by marking the onset and end points on the EEG and ECG recordings. The esophageal temperature, heart rate, respiratory rate, PETCO2, and total time of anesthesia were recorded immediately prior to initiation of the euthanasia technique. Subjective data were also recorded for occurrence of feather erection, gaping of the beak, or other body movements following initiation of TC or IPT.

Data distributions were assessed with the Shapiro-Wilk test. Several of the measured end point times were not consistent with a normal distribution, even after logarithmic transformation. Therefore, summary statistics were presented as median and first and third quartiles (calculated by inversion of the empirical distribution function), and nonparametric statistical tests were used. Values >1.5 times the interquartile range above and below the median were indicated on box plots, but were not excluded from data analysis.

<table>
<thead>
<tr>
<th>Species and variable</th>
<th>TC</th>
<th>IPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>1st–3rd quartile</td>
</tr>
<tr>
<td><strong>Spitrous</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to end point (s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cessation of pulse</td>
<td>0.0 (0.0–0.0)</td>
<td>18.5 (15.0–22.0)</td>
</tr>
<tr>
<td>Apnea</td>
<td>13.5 (1.0–17.0)</td>
<td>26.0 (18.0–32.0)</td>
</tr>
<tr>
<td>Isoelectric EEG</td>
<td>19.0 (13.0–36.0)</td>
<td>6.0 (5.0–17.0)</td>
</tr>
<tr>
<td>Abnormal ECG</td>
<td>87.3 (59.2–175.7)</td>
<td>30.0 (14.9–223.5)</td>
</tr>
</tbody>
</table>

| **Starlings**        |                |                 |                |                 |         |
| Time to end point (s)|                |                 |                |                 |         |
| Cessation of pulse   | 9.5 (0.0–10.0) | 151.0 (80.0–178.0) | 0.004          |                 |         |
| Apnea                | 9.0 (7.0–10.0) | 12.5 (4.1–17.0)  | 0.647          |                 |         |
| Isoelectric EEG      | 88.5 (27.0–128.0) | 77.5 (44.0–146.0) | 0.873          |                 |         |
| Abnormal ECG         | 103.9 (98.2–136.1) | 360.0 (227.8–485.1) | 0.025         |                 |         |

Time to each end point was measured from the initiation of the indicated method (the moment when TC was started or the beginning of injection for IPT). The P values reflect results of comparison between treatment groups by Wilcoxon-Mann-Whitney rank sum test for each end point. Values of P < 0.05 were considered significant.
sis except for comparison as otherwise noted. Data from starlings and sparrows were analyzed separately because species differences in drug metabolism and body size could be expected to affect time intervals for each euthanasia technique. Comparisons of time intervals were made between the TC and IPT groups for each species with the Wilcoxon-Mann-Whitney rank sum test, including calculation of confidence intervals for the difference in median values between groups. The Tukey method was used to define outliers for the time interval data. The proportion of birds with and without coelomic hemorrhage identified at necropsy was compared between the TC and IPT groups with the Fisher exact test. Values of \( P < 0.05 \) were considered significant. All analyses were performed with statistical software.

**Results**

From the time of initiation of each euthanasia method, variables of interest were recorded for each bird, and summary statistics were calculated for each group (Table 1). Both euthanasia techniques resulted in rapid conversion to an isoelectric EEG, and there was no significant difference in time from initiation of the euthanasia method to isoelectric EEG between TC and IPT groups for either sparrows (\( P = 0.199 \)) or starlings (\( P = 0.873 \); Figure 4). One sparrow in the TC group had a prolonged time to isoelectric EEG (> 4 times the interquartile range for this group); there was still no significant (\( P = 0.360 \)) difference in time to conversion to isoelectric EEG between methods for sparrows when this data point was excluded. Examples of EEG changes in each species were provided (Figures 5 and 6). Breathing was detectable (by capnography and observation of excursions on recorded video) after the onset of application for the TC technique in 4 of 6 sparrows and 6 of 6 starlings. The exact time of apnea could not be determined for 1 starling euthanized with TC, despite careful review of the capnographic data and video images.

**Figure 5**—Representative EEG and ECG recordings from a sparrow during anesthesia maintained by propofol administration prior to euthanasia (A), during euthanasia by IPT with propofol discontinued (the solid vertical line far left indicates the onset of the euthanasia method; B), and at the cessation of all EEG activity (vertical line labeled end; C). Seventeen seconds elapsed between the times indicated by the vertical bars. Notice the transition from continuous EEG to burst suppression to isoelectric EEG. See Figures 1 and 2 for key.
Apnea occurred prior to conversion to isoelectric EEG for all starlings, with no significant \( (P = 0.715) \) difference between groups (median intervals, -57.0 seconds for TC and -61.5 seconds for IPT). For sparrows, the timing of apnea relative to conversion to isoelectric EEG was not consistent in the TC group, whereas apnea occurred shortly after conversion to isoelectric EEG in the IPT group; there was no significant difference between groups when the 1 severe outlier was excluded (median intervals, -6.5 seconds for TC and 13.5 seconds for IPT; \( P = 0.068 \)).

The time from initiation of euthanasia to cessation of arterial pulse detection by the Doppler method was significantly shorter for the TC group, compared with that for the IPT group, among sparrows \( (P = 0.003) \) and starlings \( (P = 0.004; \text{Figure } 4) \). One starling in the IPT group that received the 100-mg/kg dose had a prolonged time to cessation of pulse (> 5 minutes), although the value was not a statistical outlier. The difference in time to cessation of pulse between euthanasia techniques for this species remained significant \( (P = 0.006) \) when this bird was removed from the analysis. Cessation of pulse occurred simultaneously with or preceded apnea in all sparrows, with no significant \( (P = 0.197) \) difference between groups (median intervals, -11.5 seconds for TC and -1.0 second for IPT). For starlings, cessation of pulse also occurred prior to apnea in 4 of 5 birds euthanized by TC for which time to apnea could be determined, whereas apnea occurred prior to cessation of pulse in all starlings euthanized by IPT, resulting in a significant \( (P = 0.014) \) difference between groups (median intervals, -1.0 second for TC and 138.5 seconds for IPT). This difference remained significant \( (P = 0.021) \) when the 1 starling with prolonged time to cessation of pulse was excluded. For all birds euthanized by TC, pulses ceased prior to conversion to isoelectric EEG; the median interval was 17.0 seconds for sparrows and 76.0 seconds for starlings. For all sparrows euthanized by IPT, conversion to isoelectric EEG occurred prior to cessation of pulses, with a median interval of 10.5 seconds.

**Figure 6** — Representative EEG and ECG recorded from a starling during anesthesia maintained by propofol administration prior to euthanasia (A), during euthanasia by TC with propofol discontinued (B), and at the cessation of all EEG activity (C). Twenty-seven seconds elapsed between the times indicated by the vertical lines. Notice the transition from nearly continuous EEG (brief [< 1 second] voltage attenuations can be seen) to burst suppression to isoelectric EEG. See Figures 1, 2, and 4 for key.
Four of the 6 starlings euthanized by IPT converted to isoelectric EEG prior to cessation of pulses (median, 36.0 seconds). The remaining 2 starlings received pentobarbital doses at the high end of the range and had cessation of pulses prior to isoelectric EEG. Consequently, there were significant differences between the TC and IPT groups for the interval between cessation of pulses and isoelectric EEG among sparrows ($P = 0.010$) and starlings ($P = 0.004$). The difference remained significant ($P = 0.018$) for starlings when the 1 bird with prolonged time to cessation of pulses was removed from analysis.

Electrocardiographic activity, although frequently with an abnormal rate or rhythm, continued in all birds after conversion to isoelectric EEG and past the absence of a detectable Doppler pulse signal (Table 1). After administration of IPT and onset of ventricular fibrillation, a regular cardiac rhythm reoccurred in 1 sparrow for 2 minutes, followed by asystole. The remaining birds did not have recovery of normal ECG complexes after the onset of any ECG abnormality (arrhythmia, fibrillation, loss of ventricular complexes, or asystole). There was no significant ($P = 0.337$) difference in the time to onset of abnormal ECG between the TC and IPT groups for sparrows, but the onset of abnormal ECG was significantly ($P = 0.025$) more rapid for starlings euthanized by TC than for those euthanized by IPT.

For each species, 4 of 6 birds in the TC group had $F_{iO_2}$ maintained at $\leq 0.40$ prior to euthanasia, and 2 of 6 had $F_{iO_2}$ maintained at $\geq 0.70$. There were no significant differences between birds that had low and high $F_{iO_2}$ for the time from initiation of euthanasia to the time of apnea (sparrows, $P = 0.814$; starlings, $P = 0.564$), cessation of Doppler pulse detection (sparrows, $P = 0.480$; starlings, $P = 0.654$), onset of isoelectric EEG (sparrows, $P = 0.355$; starlings, $P = 0.355$), or onset of abnormal ECG (sparrows, $P = 0.355$; starlings, $P = 0.643$).

In addition to time intervals between events, physiologic and anesthesia-related variables were compared between groups. There were no significant differences in esophageal temperature (sparrows, $P = 0.521$; starlings, $P = 0.109$), heart rate (sparrows, $P = 0.245$; starlings, $P = 0.868$), respiratory rate (sparrows, $P = 0.748$; starlings, $P = 0.810$), $P_{ETCO_2}$ (sparrows, $P = 1.0$; starlings, $P = 0.262$), or total time of anesthesia prior to initiating euthanasia (sparrows, $P = 0.053$; starlings, $P = 0.688$) between the IPT and TC groups.

During the first 45 seconds of TC, feather erection followed by rapid relaxation of the feathers occurred in 3 of 6 sparrows and 2 of 6 starlings, and gaping of the beak occurred in 1 of 6 sparrows and 2 of 6 starlings. During infusion of pentobarbital, muscle movements occurred in 2 birds, including stretching of the wings in 1 sparrow and toe curling in 1 starling.

**Gross pathological changes**

Pathological findings were reviewed for the 6 birds/species/treatment group used for evaluation of euthanasia end points, as well as for the additional 6 birds captured and euthanized at the conclusion of the study. Among birds euthanized by TC, 9 of 10 sparrows and 5 of 7 starlings had grossly visible coelomic, pericardial, or perihepatic hemorrhage...
Histopathologic changes

Histologic evaluation of 5 sparrows (4 and 1 from the TC and IPT groups, respectively) revealed that blood was present in various amounts around the heart base, between the caudal vena cava and the liver, and beneath the liver capsule in all birds of the TC group. Blood was also variably present surrounding the kidneys, gonads, spleen, proventriculus, lungs, or airways, but hemorrhage was not associated with parenchymal trauma in those organs and likely represented suffusion from the caval or atrial rupture sites. The liver was congested in 2 birds of the TC group, and 1 other bird in this group had congested pulmonary veins, consistent with obstruction of venous return to the heart at the time of death. In the bird euthanized by IPT, the heart and larger blood vessels were distended by coagulated blood, consistent with effects of pentobarbital injection, but there was no hemorrhage or vascular congestion present. There were also no rents or other penetrating defects in the wall of the heart or in the large blood vessels.

Ruptures of the vena cava had been identified grossly in 2 of the sparrows from the TC group that were subsequently prepared for histologic examination (Figure 7). The rupture sites were identified in the histologic sections and had attenuation of muscle fibers and hemorrhage dissecting through the vascular walls, atrial walls, or both, consistent with perimortem occurrence rather than artifactual damage to the fixed tissue. Full-thickness defects were not identified histologically in the 2 other sparrows of the TC group that did not have grossly apparent ruptures, even with stepwise sections performed to examine the area more extensively. However, in both birds, hemorrhages dissected partially through the walls of the caudal vena cava or right atrium. Both birds also had a pattern of suffusive hemorrhage similar to that seen in birds with identified ruptures. This pattern of hemorrhages suggested that cardiovascular ruptures occurred in these birds as well, although they were not represented in the histologic sections.

Discussion

In the present study, performed with passerine birds under a light plane of general anesthesia to compare TC and IPT for euthanasia, conversion to an isoelectric EEG was rapid in both sparrows and starlings, with similar time intervals measured from initiation of euthanasia for the 2 methods within each species. The isoelectric EEG pattern was continuously recorded for several minutes and appeared to be irreversible, with no recovery of EEG activity. This was used as an indicator of cessation of normal electrical activities of the brain and, in combination with irreversible apnea and cessation of arterial pulse detection by Doppler methods, was used to define death in this study. Determination of death after barbiturate infusion in other species often includes the absence of brainstem reflexes such as corneal and palpebral reflexes, but these variables could not be assessed in our study owing to the use of propofol anesthesia.

Cessation of pulse is an essential component for determination of death. In both sparrows and starlings,
TC resulted in a more rapid cessation of pulse than did IPT. Digital compression directly over the heart for ≥60 seconds in the TC technique led to rapid, and in some cases immediate, pulse cessation, which occurred prior to conversion to isoelectric EEG in all birds. The cessation of pulse was likely due to the loss of effective cardiac contractions and, in most cases, rupture of the vena cava or atrium, leading to rapid loss of blood circulation. No return of pulses was detected after digital pressure was released from the thorax. Decreased blood flow to the brain creates tissue hypoxia, which rapidly leads to an isoelectric EEG, as reflected by these results. Conversely, the IPT in this study resulted in isoelectric EEG before the loss of cardiac function and cessation of pulse in 6 of 6 sparrows and 4 of 6 starlings. This finding was expected because of the known anesthetic effects of pentobarbital sodium and findings in horses euthanized by overdose of the same drug in another study.14

The time from initiation of euthanasia to an abnormal ECG or asystole was extremely variable with both methods used in the present study. For both methods in both species, the change in ECG occurred after conversion to isoelectric EEG and after cardiac output had ceased, as evidenced by the lack of Doppler detection of the arterial pulse. Similar findings in the aforementioned study15 of horses supported the proposal that cardiac death occurs earlier and that ongoing ECG activity represents ineffective contraction with no cardiac output (electrical-mechanical dissociation) as the remaining cardiac muscle ATP is used. The presence of cardiac electrical activity does not imply effective cardiac pumping because the ECG is only a 2-D recording at the body surface of electrical fields generated by the heart, and it does not reflect the mechanical status of the heart.8,15

The time from initiation of euthanasia to apnea in birds was short (median time, ≤ 26 seconds for sparrows and ≤ 12.5 seconds for starlings) for both euthanasia methods in the present study, with no significant differences between methods for either species. In mammals, barbiturates depress the CNS beginning with the cerebral cortex, resulting in loss of consciousness that progresses to anesthesia. With an overdose, deep anesthesia progresses to apnea due to depression of the respiratory center, and this is followed by cardiac arrest.1 Because birds were under anesthesia at the onset of both euthanasia techniques in the present study, it cannot be determined whether loss of consciousness would precede or follow apnea with either technique if used on an awake bird. Apnea occurred prior to conversion to isoelectric EEG in all starlings regardless of euthanasia method. The relative timing of apnea and conversion to isoelectric EEG was less consistent in sparrows, but there was no significant difference between groups for either species. Cessation of pulse was simultaneous with or preceded apnea in all birds euthanized by TC. Therefore, it is unlikely that apnea can be considered the cause of death with TC.

The AVMA-approved method to euthanize conscious birds is IV injection of pentobarbital, and TC has historically been used as a field technique to provide a rapid death of small birds. For the present study, we elected to use anesthetized birds under controlled experimental conditions to provide instrumentation and monitoring necessary to obtain accurate physiologic end point data for each technique and to reduce covariables in determination of similarities and differences for these 2 euthanasia techniques. As such, this study provides relevant and previously unavailable information about time to loss of brain activity and death; however, the study was not designed to investigate the aspects of pain or distress associated with either treatment. Since it is now established that the times to death with TC are similar to those for the use of pentobarbital, further studies could evaluate biomarkers of distress in birds that had not been anesthetized.

The variations in times from initiation of euthanasia by IPT or TC for the variables of interest in this study might have been affected by several factors such as the sizes of birds, depth of anesthesia at the time of euthanasia, variability in dose and rate of pentobarbital injections, or investigator who performed TC. Another variable among birds was the period of time required after discontinuation of inhalation anesthesia until titration to a light plane of anesthesia with propofol, because the determining factor for initiation of euthanasia was maintenance of a continuous EEG pattern free of burst suppression. A fixed volume of pentobarbital was delivered to sparrows because the birds were similar in body size, which created a small range of doses. In contrast, starlings received a dose calculated according to body weight,12 but the dose was increased from the lower dose of 100 mg/kg (delivered to the first 2 starlings) to the higher dose of 220 mg/kg, both within the recommended range, because 1 bird given the lower dose had a prolonged time to cessation of pulse, despite a rapid conversion to isoelectric EEG. All TC procedures were performed by 2 investigators (AE and IEE) who used the same technique and had similar levels of experience with the method, and we consider it unlikely that differences in application of TC contributed to the variations in time intervals.

Because the study required death of the birds, the sample size was chosen with the goal of minimizing the number of animals used to obtain meaningful results. To evaluate the risk of type II error due to low sample size or data variability, confidence intervals were calculated for the difference in medians between treatment groups for each end point that did not have a significant effect in the rank sum test (data not shown). A true effect size of zero falls within the confidence interval for any test in which the null hypothesis cannot be rejected. For time to apnea and time to onset of isoelectric EEG in sparrows, the confidence intervals were asymmetric around 0, such that a moderate decrease in the interval width
could reveal a significant effect. This asymmetry did not prove a type II error but suggested that these variables might warrant further study. For the time to onset of abnormal ECG in sparrows, time to apnea in starlings, and time to onset of isoelectric ECG in starlings, the confidence intervals were symmetric around zero, and the risk of type II error was considered to be low.

When used in a field setting, TC is performed without supplemental oxygen administration (ie, at an FiO2 of 0.21). In the present study, anesthesia was induced with anesthetic gas in oxygen, and a ventilator was used as needed for respiratory support. Because of concerns that artificially high FiO2 and, therefore, blood oxygenation would affect the time when vital signs would be sustained after euthanasia, FiO2 was lowered after induction of anesthesia. As a result of variability in anesthetic time, an FiO2 of 0.21 was not reached for all birds prior to initiation of euthanasia. However, for both sparrows and starlings, there was no significant difference in the time from initiation of euthanasia to any of the defined monitoring end points between birds that had an FiO2 ≥ 0.40 and those that had an FiO2 ≥ 0.70 at the time TC was initiated.

During the first 45 seconds of TC, feather erection and relaxation were observed in 3 of 6 sparrows and 2 of 6 starlings but did not occur in any of the birds (6 birds/species) that had IPT. Sudden feather erection has been anecdotally associated with cardiac arrest or reduced cardiac blood flow of anesthetized birds. It has also been noted to occur in poultry killed with CO2 and in euthanasia of turkeys during the tonic phase of convulsions, and the implication of feather erection during euthanasia needs further exploration. Beak gaping and nonpurposeful body movements have been noted to occur in other avian euthanasia studies. These are considered signs of distress when they occur prior to recumbency but are considered reflexive when EEG activity and brainstem reflexes are absent.

In the present study, the occurrence of beak movements relative to the EEG pattern was inconsistent in the 3 birds for which it was observed, and the small numbers made interpretation difficult.

The most common postmortem finding unique to birds euthanized by TC was hemorrhage in the coelomic cavity, primarily between the heart and the cranial margin of the right liver lobe. Dissection of formalin-fixed specimens confirmed the source of hemorrhage was from a rupture of the right atrium or vena cava, often at the junction of the caudal vena cava and the right atrium. Microscopic examination of tissues from 4 sparrows euthanized by TC revealed pericardial hemorrhages that dissected under the serosal surfaces of the caudal vena cava and beneath the liver capsule, supporting the conclusion that rupture of the caudal vena cava or right atrium had occurred. Slides of tissues from 2 of the 4 sparrows included sections through avulsions of the right atrium or the proximal segment of the vena cava. The margins of the defects had frayed edges, with attenuation of myofibers and small amounts of intramural hemorrhage. Three of the 17 birds necropsied after TC did not have gross evidence of coelomic hemorrhage, indicating that, although rupture of the atria or vena cava frequently occurred secondary to compression of the heart, it was not essential for euthanasia. The TC technique involved direct pressure application over the heart, leading to obstruction of venous return and stopping cardiac output. The rapid cessation of pulse and apnea that occurred during TC of birds in the study was consistent with this interpretation.

The results of TC in birds of this study provided information to support that this commonly used term is misleading and that the terminology promotes a misconception that suffocation is the cause of death. The appropriate TC technique involves digital pressure directly over the location of the heart, with 2 fingers placed on either side of the body, dorsal to the pectoral muscles, where the thoracic body wall is thin and very pliable. Postmortem evidence in combination with physiologic events indicated that the heart was directly compressed, often leading to rupture in the thin-walled regions of the vena cava or atrium. For these reasons, the authors contend that the technique would be more appropriately described as rapid cardiac compression.

In addition to the comparison of physiologic end points for euthanasia by TC and IPT, the gross and histologic postmortem findings provided insights into the physical cause of death following TC. The knowledge gained in this study can be used to assist institutional animal care and use committees and researchers in further assessment of the appropriate methods of euthanasia for small birds.

Acknowledgments

Supported by the University of California-Davis School of Veterinary Medicine Ornithological Council Parrot Wellness and Welfare Research Fund. Additional funding and field logistics were provided by the University of California-Davis Museum of Wildlife and Fish Biology. The authors declare that there were no conflicts of interest.

The authors thank Barry R. Tharp, MD, for assistance with EEG interpretation; Chris Conard, Sacramento Regional County Sanitation District Bufferlands; and John Anderson, Hedgerow Farms, for providing access to properties and assistance in the field.

Footnotes

a. Kaytee Products Inc, Chilton, Wis.
b. Piramal Healthcare Ltd, Bethlehem, Penn.
c. BD Insyte, Franklin Lakes, NJ.
d. Teleflex Medical, Research Triangle Park, NC.
e. Hallowell EMC, Pittsfield, Mass.
f. GE Healthcare BioSciences, Pittsburgh, Penn.
g. Parks Medical Electronics Inc, Aloha, Ore.
h. Nihon Kohden Inc, Irvine, Calif.
References


