

# Efficacy of a Novel Electrical Shock Trap for Pest Control Programs

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An effective rodent pest control program is an important aspect of any animal care and use program. It ensures the SPF status of rodent colonies and also protects human safety as regards zoonoses prevention. According to the *AVMA Guidelines for Euthanasia of Animals*, kill traps do not always render a rapid or stress-free death, and thus, the use of live traps followed by euthanasia is preferred; however, they also state that, although newer technologies improve kill trap performance by assuring rapid loss of consciousness, individual testing of traps is recommended to ensure the device works properly. Here, we evaluated an electrical shock trap as an option for vermin control in animal facilities. We assessed the trap's ability to quickly induce irreversible loss of consciousness and death with minimal pain and distress. This was performed by placing a modified trap (allowing visualization of the animal's interaction within the trap) in a test chamber and allowing animals to freely interact with the trap. Assays were videotaped by an overhead and a side camera. We measured time to induce loss of consciousness and time to death using male ( $n = 10$ ) and female ( $n = 10$ ) Crl:CFW(SW) mice. A subset of electrical shock ( $n = 10$ ) and CO<sub>2</sub> ( $n = 4$ ) euthanized animals were used for blinded comparative necropsy and histopathology. Our results indicate that the trap has a 100% kill rate. Mean time to unconsciousness was  $7.35 \pm 3.76$  s, while mean time from unconsciousness to death was  $25.62 \pm 7.2$  s. Histopathology revealed a 20% (2/10) occurrence of focal mild dermal lesions, indicative of perimortem burn injury, in the electrical shock animals. No other histologic changes associated with electrocution were identified. In conclusion, this system presents a viable alternative to current mouse traps, while improving animal welfare compared with other kill trap options, as well as allowing reduced labor investment associated with pest control management.

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## Introduction

An effective and robust pest control program is an essential aspect of an animal care and use program. Specifically, wild/feral and escaped rodent control prevents exposure of rodent colonies to adventitious pathogens and of personnel to zoonotic agents. A review of the literature indicates that wild and feral rodents captured in urban centers and laboratory vivariums around the world serve as common vectors of zoonotic diseases and excluded pathogens in SPF rodent vivaria, as well as sources of allergens and significant financial loss.<sup>1–8</sup>

There are many commercially available rodent traps, and they are classified as either live or kill traps. Live traps capture animals allowing for relocation or humane euthanasia, whereas kill or lethal traps aim to kill the trapped animals acutely.<sup>1–3</sup> Regardless of the classification or mechanism of action, their efficacy and humaneness need to be evaluated before use in animal facilities.<sup>4,5</sup> There exists a dearth of information with respect to standardizing the evaluation of rodent traps in most countries, including the United States. The European Union working group Non-Chemical Alternatives for Rodent Control (NoCheRo) was tasked with creating such guidance in response to changing regulations regarding anticoagulant rodenticides.<sup>6</sup> Efficacy is regarded as the trap's ability to capture a rodent and to reliably perform over time regardless of the rodent size. The capture can be enhanced by the placement of baits such as food, chemical attractants, or pheromones. Meanwhile, humaneness

pertains to ensuring animal welfare, especially assessing animal well-being and effective management of pain and distress.<sup>5</sup> The *Guide for the Care and Use of Laboratory Animals* (the *Guide*) states that if traps are used, methods should be humane; traps that catch pests alive require frequent observation and humane euthanasia after capture.<sup>5</sup> The *Guide* also states that all animals should be observed for signs of illness, injury, or abnormal behavior by a person trained to recognize such signs, and as a rule, such observation should occur at least daily; and in this regard, the *Guide* makes no distinction between animals that are maintained for research, teaching and testing, versus those that may be confined within a trap.<sup>5</sup> Meanwhile, the *AVMA Guidelines for Euthanasia of Animals* (*AVMA Guidelines*) indicate that while kill traps do not consistently meet the Panel on Euthanasia's criteria for euthanasia, such traps can be practical.<sup>4</sup> The *Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research* indicate that lethal traps should result in a clean, effective kill and should be checked at least once a day, and in the event that an animal is still alive, it should be immediately dispatched in accordance with the *AVMA Guidelines*.<sup>4,7</sup>

Recent advances in technology have provided tools that can be integrated into pest control programs to meet regularity requirements while minimizing the required labor of daily monitoring of rodent traps and eventual euthanasia of trapped rodents.<sup>3,8,9</sup> These include but are not limited to the use of lithium-ion batteries, Bluetooth, low-frequency radio waves, cellular networks, cloud storage, and mobile notifications through applications. The *AVMA Guidelines* state that although newer technologies are improving kill trap performance to achieve loss of consciousness quickly, individual testing is recommended to be sure the trap is working properly.<sup>4</sup> Electrocution as a means

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of euthanasia is acceptable with conditions that ensure sufficient electric current first passes through the brain to induce loss of consciousness and tonic and clonic epileptic spasms.<sup>4</sup> Effective electrocution includes loss of eyeblink and moving object tracking, opisthotonos, tonic spasm progressing to clonic spasms finally resulting in muscle flaccidity.<sup>4</sup> As per the *AVMA Guidelines*, unconsciousness can be induced by any acceptable method including passage of electric current through the brain.<sup>4</sup> These guidelines provide specifics for a number of vertebrate species, including cattle, pigs, and sheep but no information for requirements in mice. Here, we evaluated the use of a commercially available electronic shock trap regarding its efficacy to induce irreversible loss of consciousness and eventual death with minimal pain and distress.

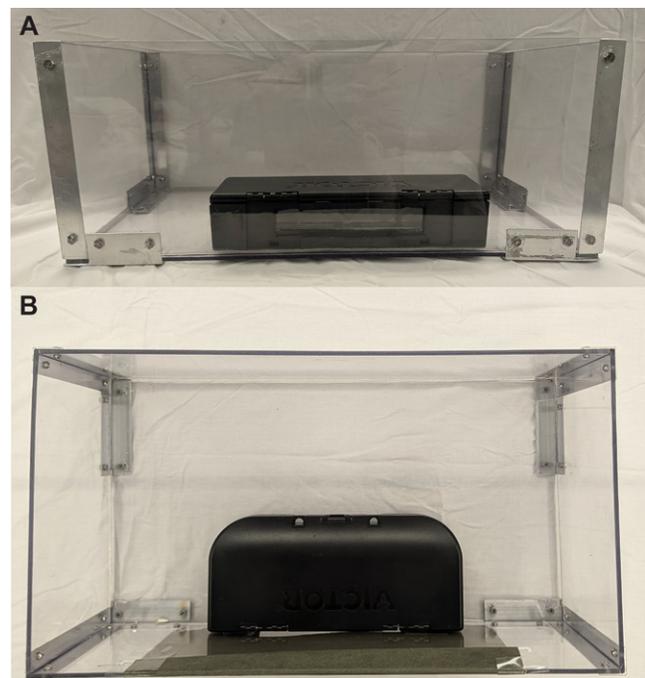
**Ethical review.** The animal care and use program at Johns Hopkins University (JHU) is accredited by AAALAC International, and all animals are maintained in accordance with the recommendations provided in the *Guide*.<sup>5</sup> This study was approved by JHU's IACUC in agreement with the AALAS position statements on the Humane Care and Use of Laboratory Animals and Alleviating Pain and Distress in Laboratory Animals.<sup>10</sup>

## Materials and Methods

**Housing and husbandry.** All rooms were maintained at 68 to 77 °F, 30% to 70% RH, ventilation of 10 to 15 ACH, and 14:10-h light:dark cycle. Mice were housed in individually ventilated cages (EM500 for mice; Tecniplast, Buguggiate, Italy). Cages with 1/4-in. corncob bedding (ENVIGO Teklad™ 1/4" Corncob Bedding; 7097; Madison, WI), a single cotton nestlet (Ancare Nestlets™; NES3600, Bellmore, NY) for enrichment, and autoclavable feed (ENVIGO Teklad Global 18% Protein Extruded Rodent Diet; 2018SX; Madison, WI) were autoclaved and changed every 2 wk under a cage changing station (AG4 Animal Transfer Station; The Baker Company, Sanford, ME). Reverse-osmosis-treated water was either hyperchlorinated or UV-treated before distribution to cages via automated watering systems (Avidity Science, Waterford, WI). Colony mice were monitored quarterly for pathogens via serology and PCR of soiled-bedding sentinel mice and exhaust air duct PCR. Excluded pathogens included Sendai virus, pneumonia virus of mice, mouse hepatitis virus, minute virus of mice, mouse parvovirus 1 and 2, Theiler murine encephalomyelitis virus, reovirus, epizootic diarrhea of infant mice, lymphocytic choriomeningitis virus, ectromelia virus, murine adenovirus I and II, murine cytomegalovirus, *Mycoplasma pulmonis*, fur mites (*Myobia*, *Myocoptes*, and *Radfordia* spp.), and pinworms (*Aspicularis* and *Syphacia* spp.).

**Animals.** Twenty-four adult Swiss Webster (CrI:CFW[SW]) (12 male and 12 female) were used, varying in age from 7 to 72 wk (mean: 15.8 wk) and in weight from 21.4 to 55.6 grams (mean 38.7 g). All were either foster litters, retired breeders from our rederivation core, or retired from other protocols involving non-invasive procedures. Twenty animals (10 male and 10 female) were used to evaluate the shock trap, and 4 animals (2 male and 2 female) were CO<sub>2</sub> controls for necropsy and histopathology. Animal numbers were based on comparable publications in the field<sup>11,12</sup> and recommendations of the NoCheRo guidance documents.<sup>6</sup>

**Study design.** A test chamber was made of acrylic and aluminum (24-in. width × 12-in. depth × 8-in. height) with the trap positioned along the long axis to allow for video and direct visual recording of the animal's interaction with the trap (Figure 1). Animal demographic information (age, sex, and weight) was recorded. Animals were placed into the chamber

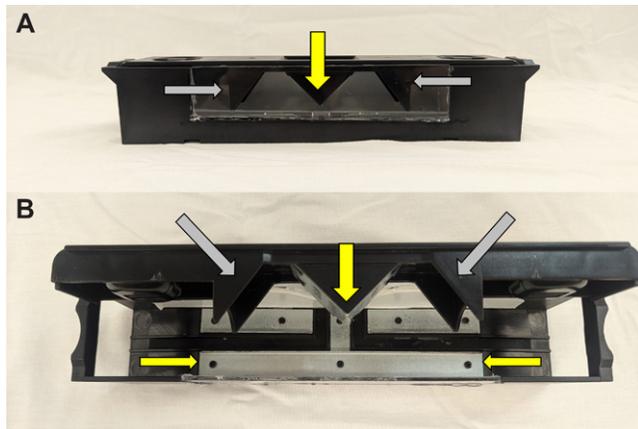


**Figure 1.** (A) Front view of the test chamber with the trap aligned along the long axis against the wall. The viewing window allowing for visualization of the interaction of the mouse with the trap. (B) Top down image of the electrical shock trap positioned for use in the test chamber. Trap is positioned to utilize the natural thigmotaxic behavior of the mouse.

and allowed to interact freely with the trap. Animals were not habituated to the trap to simulate real-world scenarios of escaped or vermin mice encountering the trap. Traps were not baited to not bias the animal's interaction with the device. Once the animal entered the trap and the electrocution mechanism was triggered by the animal, observers recorded time to unconsciousness, cessation of respiratory rate, and death. Death was confirmed by auscultation immediately after the shock cycle had ended. This was followed by cervical dislocation for those animals not submitted to necropsy. CO<sub>2</sub>-control animals were euthanized in euthanasia chambers and gradually filled with CO<sub>2</sub> at a flow rate of approximately 50% of chamber volume per minute.

**Rodent trap.** Rodent traps ( $n = 2$ ; Victor V-link™ mouse tunnel trap; Woodstream Corporation, Lancaster, PA) were modified by the vendor to allow for visualization of the interaction of the mouse and the trap (Figure 2). This was done by replacing a portion of the tunnel casing with transparent acrylic. All manufacturer instructions were followed in trap setup and use. The electrical shock trap used in this study had a removable tunnel that was 4.1 cm wide, 3.5 cm tall, and 25 cm long. The tunnel had an inverted triangular metal top plate that was 5 cm long, and the center of the trap was directly in the middle at 2.5 cm. There were 3 metal floor channels parallel to and 1 cm apart from each other: all were 1.4 cm wide and 0.25 cm deep each; one was 16.5 cm long while 2 others were 6.4 cm long.

**Video recording.** Video cameras were positioned in front of (Nikon D5200 24.1 MP CMOS Digital SLR; Minato City, Tokyo, Japan) and above (Lorex D24281B-2NA4-E, Linthicum Heights, MD) the test chamber to record the interaction of the animal with the trap. Video recordings were then evaluated by a veterinarian (MW) to determine when the trap was triggered, time to irreversible unconsciousness (defined as the



**Figure 2.** (A) The modified tunnel portion of the electrical shock trap in the closed position with the viewing window installed. (B) Tunnel portion opened up with top laying at a 90° angle from the tunnel base. Yellow arrows denote location of the metal conduction plates of the trap. Grey arrows indicate the location of the baffles, which cause the mouse to contact the triggering plate and starting the high voltage electrical shock train.

point when the eyes involuntarily opened after the initial shock and no voluntary eye blink was present), and time to death (defined as the cessation of respiration and complete muscle flaccidity indicated by neutral positioning of the ear pina) (Figure 3).

**Necropsy and histopathology.** To compare the electrical shock-induced death to carbon dioxide euthanasia, a complete diagnostic necropsy was performed on 14 animals (10 shock animals and 4 CO<sub>2</sub> euthanized animals). Experimental tissue collection included the head (brain, ears, nose), thoracic tissues (esophagus, heart, thymus, trachea, lungs), forelimb and hindlimb (skin, skeletal muscle, bone, nerves), and specific skin regions (muzzle, ventral thorax, ventral abdomen). Tissues were formalin-fixed (10% NBF), processed with ethanol and xylene (Tissue-Tek VIP; Sakura), and paraffin embedded. The head, forelimb, and hindlimb (skin removed) were fixed and decalcified in Formical4 (StatLab.com). Fixed tissues were trimmed at 3 to 4 mm thick into cassettes, processed routinely to paraffin (Tissue-Tek VIP; Sakura), sectioned to 4 to 5 μm, and stained with hematoxylin and eosin. All slides were reviewed by 2 veterinary pathologists (KC, CB), blinded to the euthanasia method.

**Statistical analysis.** All statistical analyses were performed in Prism 10 (GraphPad, San Diego, CA). Times from triggering the trap to irreversible unconsciousness and time from irreversible unconsciousness to death, reported as means ± SD, were tested

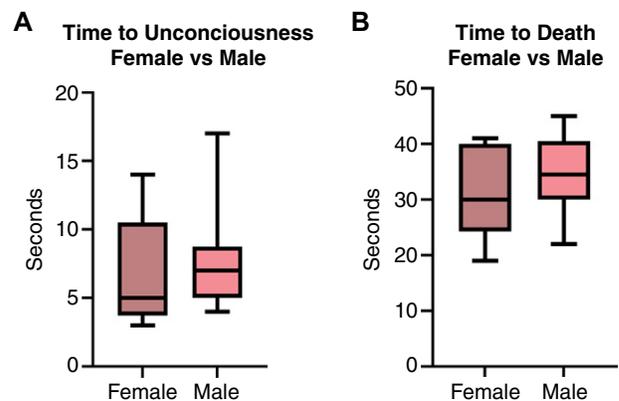
for normality and homogeneity of variance using Shapiro-Wilk tests. Unpaired *t* tests were performed to evaluate significant differences between groups. A *P* value <0.05 was considered statistically significant.

## Results

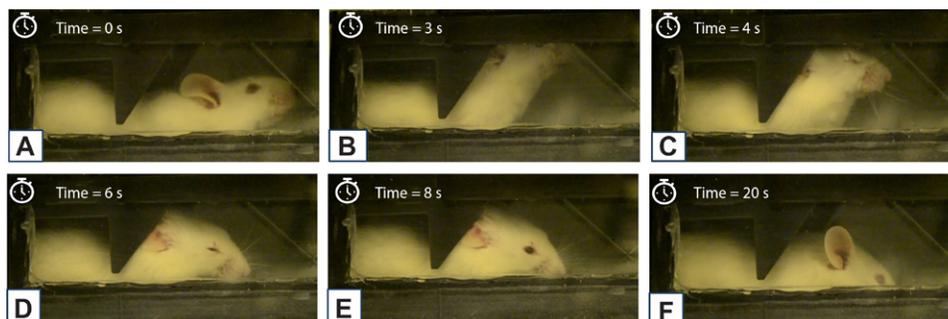
**Trap efficacy.** Traps used had a 100% success rate of producing irreversible unconsciousness and death in this cohort (*n* = 20). The average time to irreversible unconsciousness was 7.35 ± 3.76 s. The average time from unconsciousness to death was 25.62 ± 7.2 s. Between males and females, there were no significant differences between time to unconsciousness (females: 6.8 ± 3.9 s; males: 7.9 ± 3.7 s) or time to death (females: 31.3 ± 8.3 s; males: 34.6 ± 6.99 s) (Figure 4). We found no significant difference in age, sex, or weight class for time to irreversible unconsciousness or time from unconsciousness to death (Table 1).

**Necropsy and histopathology.** Gross examination identified focal erythema on the nonhaired portion of the muzzle in 4/14 mice (29%) across both groups. Histologically, 10/14 mice (71%) across both electric shock and CO<sub>2</sub> euthanasia groups (including the 4 with muzzle erythema) had mild extravasation of erythrocytes in the nasal cavity, suggesting a perimortem finding.

Pathologic evaluation comparing the novel shock trap with conventional CO<sub>2</sub> euthanasia revealed focal mild histologic changes (<3 mm in sections examined) in 2/10 mice (20%) subjected to electric shock. Changes were identified at presumed “contact sites.” Both mice had focal lesions characterized by a loss of epidermal-dermal architecture with scattered pyknotic



**Figure 4.** Results of the unpaired *t* test assuming equal variance between groups. (A) results comparing time from triggering the trap to unconsciousness between females and males showing no significant difference. *t* = 0.6462, *df* = 18, *P* = 0.5263. (B) Time to death between females and males. *t* = 0.9537, *df* = 18, *P* = 0.3529.



**Figure 3.** Still images of a mouse’s interaction with the trap. (A) Entering the trap. (B) Initial reaction after triggering the trap with dorsalflexion of the head and neck, (C) head beginning to fall and body relaxation, (D) beginning of involuntary opening of the eyes, and (E) head ventral eyes open, interpreted as unconsciousness. (F) Death, ears in neutral resting position, eyes completely open, respiration stopped, and shock cycle over. This was sequence of events was consistent across all trials.

**Table 1.** Results of unpaired *t* tests

Unpaired <i>t</i> test results	<i>t</i>	df	<i>P</i>
Time to unconsciousness by age	1.536	18	0.1419
Time to death by age	0.4149	18	0.6831
Time to unconsciousness by weight class	1.536	18	0.1419
Time to death by weight class	0.4149	18	0.6831
Time to unconsciousness by sex	0.6462	18	0.5263
Time to death by sex	0.9537	18	0.3529

Age groups were <10 weeks ( $n=10$ ) and >12 wk ( $n=10$ ), weight classes were those <35 g ( $n=10$ ) and >35 g ( $n=10$ ), and sex groups were female compared with male. No group showed any significant difference between time to unconsciousness or time to death.

nuclei and a deep basophilic matrix, indicative of perimortem burn injury (Figure 5). One animal had a focal lesion of the muzzle and the other on the ventral abdominal skin extending for 3 mm. No other histologic patterns were appreciated that were not consistent with perimortem changes.

## Discussion

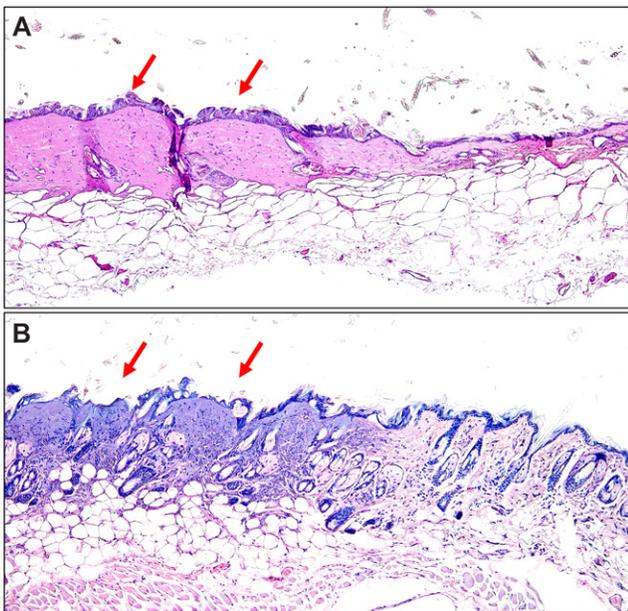
Vermin mice penetrating SPF rodent colony housing can carry adventitious pathogens like mouse parvovirus, pinworms, and fur mites. Viral pathogens typically require test-and-cull or breeding moratorium strategies, which have devastating effects on breeding colonies and research studies. Meanwhile, treatment for pinworms and fur mites requires strict quarantine procedures and the use of medications that can confound research outcomes including behavioral studies.<sup>13</sup> In addition, loose laboratory rodents can thrive in a vivarium, especially in the cage wash, where there is easy access to feed and water. Loose rodents in an animal facility can also cause physical damage like holes in the walls or in the bottoms of doors and

severed electrical and equipment wires because of the rodents' gnawing behavior.

An integrated pest management (IPM) is essential to eliminate and prevent pests. IPM is a system of managing pests that includes prevention and corrective measures.<sup>14</sup> Rodent IPM differs from traditional rodent control, as the former emphasizes prevention while the latter depends heavily on rodenticide application.<sup>14</sup> IPM aims to determine the causes of the problem by thoroughly inspecting the facility before starting control. Some pertinent questions include what are they feeding on, where are they nesting, where did they come from.<sup>14</sup> In our experience, IPM also includes surveillance and monitoring, not only for the presence of pests but also for pathogens that the pests may carry. For example, if a mouse is trapped, it is advisable to identify, if possible, whether it is vermin or an escaped colony mouse based on factors such as appearance and presence of identification markers like an ear tag or an ear notch. Testing the trapped animal for the facility's exclusion pathogen list is also recommended especially if the facility has ongoing outbreak issues with infectious agents. Once a loose rodent is found in the animal facility, all efforts should be made to identify breaks in biosecurity to mitigate issues and risks, especially disease outbreaks.

The use of rodent control methods is, thus, an essential part of pest management programs. Rodenticides and other chemicals like liquid bait and tracking powders are typically not used in animal facilities because of the potential toxicity to colony animals. Meanwhile, the primary physical control used against rodents is trapping. Traps offer significant advantages over rodenticides including the absence of environmental contamination and toxicity to colony animals. Traps can be categorized as either live or kill traps. Live traps are considered the more humane of the 2, but they require significant investment of labor in the form of daily monitoring to prevent potential animal distress related to food or water deprivation, and euthanasia of captured animals. However, it is of note that AAALAC, International indicates that the frequency of monitoring traps can be determined by the IACUC if the traps have food and water.<sup>15</sup> Live traps are typically used for catch-and-release methods. In the animal facility, rodents trapped in live traps are typically euthanized, most likely with the same euthanasia equipment (for example, CO<sub>2</sub> stations) used for colony animals. In this regard, it is important that such equipment be decontaminated after each use to prevent pathogen transmission. Meanwhile, traditional kill traps such as glue traps and snap traps have the potential to cause significant animal welfare concerns. Snap traps are typically made of wood, metal, or plastic with a killing bar that is powered by a spring and is released when a rodent steps on the trigger, killing the rodent by breaking its neck, skull, or back.<sup>14</sup> Snap traps can malfunction, especially because of factors such as the size of the trapped animal and size of the trap, such that it does not cause an instant kill or an animal's extremities can be amputated or mutilated. Glue traps have a layer of malleable mixture that traps rodents by sticking to the animal's extremities and body, causing it to struggle, which will lead to exhaustion and dehydration, eventually causing death.

The AVMA *Guidelines* categorizes electrocution as acceptable with conditions and is considered humane if the animal is first rendered unconscious.<sup>4</sup> The conditions include personnel proficiency and appropriate equipment that allows passage of sufficient current through the brain to induce loss of consciousness and tonic and clonic epileptic spasms.<sup>4</sup> Induction of unconsciousness is an important step before or concurrent with, not after, cardiac fibrillation. An advantage of electrocution is that it does not contaminate tissues.<sup>4</sup> Disadvantages



**Figure 5.** Photomicrograms of the hematoxylin and eosin stained slides from the 2 lesions found in electrical shock animals (2/10) submitted for necropsy and histology. A is 10× magnification of the focal lesion (red arrows) found on the abdominal skin approximately 3 mm in length. (B) 10× magnification of the focal lesion found on the muzzle (red arrows). Both are characterized by a loss of epidermal-dermal architecture with scattered pyknotic nuclei and a deep basophilic matrix, indicative of perimortem burn injury.

include that it can be ineffective in dehydrated animals and that it is aesthetically objectionable because of violent extension and stiffening of limbs, head, and neck.<sup>4,16</sup> In our study, we observed dorsiflexion of the head and neck, but no extension or stiffening of the limbs. This could be due to the confined trap design preventing significant movement of the animals after being shocked.

Unconsciousness can be defined in the context of the stages of anesthesia. One study emphasized that to evaluate the efficacy of euthanasia and anesthetic agents, it is important to understand these stages and how they correlate to conscious and unconscious responses to noxious and distressing stimuli.<sup>17</sup> Briefly, stage I is the period from induction to loss of consciousness. Stage II is the period from loss of consciousness to response to stimuli. Stage III is the surgical plane of anesthesia, when amnesia, analgesia, and muscle relaxation are achieved. Stage IV is generally avoided during surgical procedures, because it involves cessation of cardiovascular responses and respiratory function; it is, however, the desired state when euthanasia is the purpose. For the animal, only the physiologic changes and behavioral responses experienced during stage I are of concern; thus, careful evaluation and interpretation of movement or response to stimulation as a conscious activity are warranted.<sup>17</sup> It has been suggested that defining unconsciousness in animals is not a simple task.<sup>17</sup> Historically, it is defined as a loss of righting reflex, also known as loss of position,<sup>18</sup> although it has been argued that this is not based on scientific evidence given that loss of reflex responses varies according to species, type of reflex, and anesthetic agent.<sup>19</sup> For our study, we defined unconsciousness as the point when the eyes involuntarily opened after the initial shock and no eye blink was present as a surrogate for loss of palpebral reflex. This was used as the palpebral reflex could not be safely evaluated due to the trap design. Dorsoflexion of the head and neck was used as a surrogate for opisthotonos due to the physical constraints of the device preventing true opisthotonos, which is expected in euthanasia by electrocution.<sup>4,20</sup>

The electric trap evaluated here is designed with lithium batteries as a power source allowing for wireless placement. Placement of the trap exploits a mouse's natural thigmotaxic tendencies, by placing the tunnel opening along walls. This trap has 2 tunnel openings so it can be accessed from either side. The floor of the trap houses 2 parallel metal plates and in the center of the roof of the trap is a third metal plate in the shape of an inverted triangle (Figure 2). The top portion of the inverted triangle also can serve as a bait station with small holes in the metal plate to allow for olfaction without ingestion of the bait. Once the mouse enters the trap, it is forced to squeeze under a baffle, which causes the mouse to angle its muzzle toward the triangular metal plate while its paws and ventrum are in contact with the 2 floor plates. The animal coming in contact with the top plate completes the circuit triggering the trap, initiating a high-voltage pulse shock train consisting of repeated shocks back-to-back with milliseconds between each shock. While the details of the shock sequence are proprietary, authors can confirm that traps used in this study do meet or exceed recommended stunning settings (amperage, voltage, and frequency) in other species where electrocution is commonly used for euthanasia.<sup>4,16</sup> The trap's shock cycle is approximately 30 to 40 s. There is a novel feedback loop to confirm that the rodent is in the trap after the shock cycle, and if no kill is detected, the trap initiates a rearm feature for reset. When the shock train is delivered and the animal is confirmed dead by the system, a low-frequency radio wave signal is sent to their software, and notification is delivered via their web and mobile

applications. The trap cannot be triggered again until it has been checked and the carcass is removed. If the trap senses that its battery levels are too low to produce and affective chock, it will alert the users and not function until the batteries are replaced. In addition, the individual traps communicate to the network daily to relay trap status and battery power levels, allowing for distance management of the trap network. Traps are rated for up to 200 consecutive kills before needing replacement of the power source. Certainly, there are other electrical shock traps that are commercially available. For example, other devices use different trigger methods such as infrared sensing to activate the shock sequence<sup>21,22</sup> or have simple 2-plate designs, requiring a trapped rodent to touch the 2 plates to complete the circuit.<sup>22</sup>

The video recordings demonstrated a consistent chronology of 4 events when an animal came into contact with trap's metal plate to trigger the mechanism: immediate eyelid closure, head and neck dorsiflexion or opisthotonos, gradual lowering of the head, and loss of voluntary eyelid control (Figure 3). In some cases, this was followed by tonic spasms progressing to clonic spasms as seen in other species when electrocution is used as a means of euthanasia.<sup>4,20,23</sup>

Our results revealed that this novel shock trap system was capable of rendering an animal irreversibly unconscious quickly, a mere  $7.35 \pm 3.76$  s, and death by another  $25.62 \pm 7.2$  s. In contrast, death by glue traps is up to 48 hours.<sup>24</sup> When compared with conventional CO<sub>2</sub> euthanasia methods, the time to unconsciousness in this shock trap is considerably faster than the time to recumbency (average  $37 \pm 7$  s) in a recent study.<sup>25</sup> CO<sub>2</sub> at 50% volume per minute displacement rate was effective in rendering euthanasia after 2 min.<sup>26</sup> Isoflurane at 5% has a time to loss of righting reflex (an analog of time to unconsciousness) of 60 to 90 s.<sup>4,27</sup> Mice induced with 5% isoflurane at 1 L/min followed by 100% CO<sub>2</sub> rendered mice euthanized in 3.9 min.<sup>28</sup> NoCheRo guidance for the evaluation of rodent traps outlines 2 categories of animal welfare and time to irreversible unconsciousness. Category A (which is considered "improved animal welfare") is defined as  $\geq 80\%$  of animals being irreversibly unconscious in 30 s.<sup>6</sup> A study<sup>11</sup> recently evaluated 2 commercially available electrocution traps and found that the traps rendered mice unconscious in  $23 \pm 3$  s or  $22 \pm 2$  s. These values were 3 times the mean times recorded in our study. Of note, that study<sup>11</sup> was not able to determine the time to death for the traps they tested but only indicated that when traps were opened, all animals that were unconscious within 120 s were already dead, except for one mouse that remained conscious after 30 s. There was not much information in the paper regarding the design and mechanism of the traps used, other than the traps had 2 metal plates on the trap base that were bridged.<sup>11</sup> In contrast, the trap we evaluated had 3 metal plates. We postulate that the design and the overall mechanism of our trap yielded better study parameters. Thus, euthanasia using our tested electrical shock trap is faster than traditional methods of rodent euthanasia and other electrical trap products on the market<sup>11,12</sup> and exceeds NoCheRo standards for improved animal welfare. It is the authors' opinion that this system does meet the criteria for rapid irreversible loss of consciousness.

The tendency of mice to enter a trap relies on several behavioral factors such as thigmotaxis and novel object recognition. The former is an important consideration for device placement; that is, the device needs to be placed close to the walls. The latter explains the innate tendency of mice to explore novel objects. To simulate a real-life scenario of vermin and loose mice in an animal facility, we did not include a habituation phase typically found in some object recognition

test protocols in our study.<sup>29</sup> We found that the interval time from placement of a mouse subject in the test box to the mouse entering the trap device varied vastly among mice, taking 1 s to 5 min; (data not shown). Some mice explored the trap device, climbing on top of it and investigating one or both entrances multiple times, while others ignored it entirely, indicating a potential neophobic response. Overall object exploration time in such behavioral assays depends on several factors, including the state and strain of the animal, and the environment.<sup>29</sup> For example, BALB/c and 129 mice typically have aplasia of the corpus callosum, making them poor candidates for learning behavior research.<sup>30</sup>

Thermal injury, hemorrhage at or near the site of electrical contact, skeletal muscle necrosis, hemolysis, thrombosis, and damage to the brain have been reported in other species euthanized or killed by electrocution.<sup>31–33</sup> Tissue collections for histologic evaluation were selected based on the anticipated mechanism of euthanasia (electrocution resulting in seizure and eventual cardiac fibrillation). In our study, there were only 2 areas indicative of perimortem thermal injury, that is, a focal region on the muzzle and ventrum with loss of epidermal-dermal architecture with scattered pyknotic nuclei and a deep basophilic matrix (Figure 5). There were no gross findings or other patterns of histopathologic changes consistent with electrocution. We postulate that the paucity of findings could be due to factors such as the short duration of contact of the animal with electricity and the large contact area size. With long contact times, more energy causes electrothermal heating of tissues and tissue destruction at the skin contact points and in inner organs.<sup>31</sup> Meanwhile, a large contact area results in less energy acting upon the tissue per square area.<sup>31</sup> The trap device has a surface area of 44.8 cm<sup>2</sup> that delivers the electrical shock, compared with a mean body surface area of 67.30 cm<sup>2</sup> for an approximately 5-month-old C57BL/6J mouse.<sup>34</sup> Finally, there were no significant findings seen on histopathology when comparing electrocuted mice to CO<sub>2</sub>-euthanized mice in our study. In contrast, microscopic atelectasis has been found in mice euthanized with CO<sub>2</sub> possibly due to the reabsorption of CO<sub>2</sub>, in comparison to mice euthanized by isoflurane or sevoflurane, while foamy efflux was observed in animals euthanized by all 3 inhalant gases.<sup>35</sup> Another study reported more severe pathologic alterations of the lungs after CO<sub>2</sub> exposure compared with isoflurane.<sup>17</sup>

A novel feature of the device tested is that it is connected to a server via low-frequency radio waves and Bluetooth connection. It promptly sends notifications via an app after the trap is triggered. All traps in place are also accounted for in a database, where one can view the status of each trap, including battery life and activity. These low-frequency radio waves pose no known risk to human or animal health as they are nonionizing inaudible frequencies.<sup>36</sup> The technology potentially eliminates the need for physically checking every trap. While the *Guide* and other guidelines indicate the need for daily monitoring of traps, the *Guide* also recognizes the concept and application of performance standards, which provide flexibility in achieving an outcome by granting discretion to those responsible for managing the animal care and use program, the researcher, and the IACUC.<sup>5</sup> In our program, the IACUC approved the monitoring of this specific electrical shock trap on a weekly basis instead of daily, though notification via the app resulted in immediate checking of that trap. Ultimately, this can result in more rapid removal of vermin and escaped mice while improving animal welfare concerns associated with the use of live traps (limited food and water resources) which rely on frequent monitoring

by animal care staff. Because of the trap's capabilities, we have deployed this product in areas outside of the vivarium like the loading dock and surrounding outdoor enclosures for nonhuman primates. These are areas where there is no access to rodent CO<sub>2</sub> euthanasia stations and bringing vermin mice to the vivarium for CO<sub>2</sub> euthanasia can pose contamination risk to SPF rodent colonies.

Limitations of this study include the reliance on only visual confirmation of the loss of unconsciousness and time of death. As an effect of this, the times of death that were recorded were a more conservative point and consistent with when the shock train had concluded. Telemetry device implantation was considered during experimental design but the potential complication of electrical interference during the shock event precluded us from pursuing this. In addition, we only tested one brand of electrical shock trap. We recognize that the use of other products requires additional studies to achieve performance standards and ensure the efficacy of the device used.

In summary, this novel electrical shock trap system provides rapid irreversible loss of consciousness and was 100% efficacious in the euthanasia of all animals tested. The system provided rapid confirmation of trap triggering via its electronic system, and no malfunctions were noted. This system represents a viable alternative to current mouse traps, while ensuring no animal welfare concerns and reducing labor investment associated with pest control management in vivaria.

## Conflict of Interest

The authors have no conflicts of interest to declare.

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