The use of soapy water and freezing as secondary steps for euthanasia in cave (*Blaberus giganteus*) and Madagascar hissing (*Gromphadorhina portentosa*) cockroaches

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OBJECTIVE
To evaluate methods for euthanizing cave cockroaches (CCs; *Blaberus giganteus*) and Madagascar hissing cockroaches (MHCs; *Gromphadorhina portentosa*). It was hypothesized that both suggested methods would be effective for humane mass euthanasia of both species.

SAMPLE
Approximately 800 CC.

METHODS
The CCs were separated into replicate groups of 25, 50, 75, 100, and 150 grams and placed into 3.8-L plastic bags. Twenty-seven MHCs were divided into groups of 2 to 3. The study took place from January to March 2023. All CC groups were exposed to 100% carbon dioxide (CO2) at a rate of 4 L/min until the bag was full. Madagascar hissing cockroaches were similarly anesthetized using either CO2 or 2 mL of isoflurane on a cotton ball in a 1-L container or a combination of CO2 and isoflurane. Once cockroaches were immobile, secondary euthanasia steps were performed. One bag of CCs per weight category was exposed to soapy water (5% Dawn dishwashing liquid), and the second was placed into a −80 °C freezer. The containers of MHCs were evenly exposed to the 2 euthanasia methods. Individuals remained in their secondary euthanasia method for 30 minutes.

RESULTS
Regardless of the weight of the CCs within each bag, there was no impact on time (1.8 ± 0.4 minutes [mean ± SD]) to immobility. The failure rates for both species were 0.2% CI (−0.1% to 1.5% [1/413]) for soapy water and 0.5% CI (0.005% to 1.9% [2/414]) for the freezer method. These results support the use of both 2-step euthanasia methods in CCs and MHCs.

CLINICAL RELEVANCE
These methods will serve as an evidence-based alternative for humane mass euthanasia in cockroaches.

Keywords: carbon dioxide anesthesia, freezing, humane death, isoflurane, soapy water
Two species of particular interest are the cave cockroach (CC; Blaberus giganteus) and Madagascar hissing cockroach (MHC; Gromphadorhina portentosa). These species present challenges as, along with other cockroach species, they are capable of surviving extreme temperatures, high drug concentrations, and even immersion in alcohol and formaldehyde. Within the guidelines from the AVMA, there are few acceptable methods for euthanasia of terrestrial invertebrates. Terrestrial invertebrates are primarily immobilized with isoflurane or carbon dioxide (CO₂). Secondary steps include injectable methods, freezing, or immersion in solutions such as formaldehyde or ethanol. Through work completed in a pilot of this study, as well as from another prospective euthanasia study, concerns with all of these methods have been identified.

Following exposure to CO₂, cockroaches remain anesthetized for 5 minutes or less, which raises concerns that they may be conscious during the secondary euthanasia step. In addition, there is evidence that CO₂ can be painful or ineffective for some invertebrates. Injectable chemical methods are effective and appear to decrease distress, but they require individual handling for administration and thus are impractical for smaller species and mass euthanasia. Injectable chemical methods as a means of euthanasia is considered inhumane in some species. Injectable chemical methods are effective and appear to decrease distress, but they require individual handling for administration and thus are impractical for smaller species and mass euthanasia. Injectable chemical methods as a means of euthanasia is considered inhumane in some species. Injectable chemical methods are effective and appear to decrease distress, but they require individual handling for administration and thus are impractical for smaller species and mass euthanasia. Injectable chemical methods as a means of euthanasia is considered inhumane in some species.

In contrast, a 2-step ethanol procedure described for euthanasia in a variety of invertebrate species appears to be straightforward and requires little training. However, there are indications that this could be painful or ineffective for some invertebrates. This method was ineffective for MHCs in a pilot of this study (unpublished data by authors). This contrasts with findings from a study that evaluated multiple euthanasia techniques in mealworms, where immersion in ethanol was 100% effective in euthanizing them. This variation highlights the importance of species-specific euthanasia studies. Another technique evaluated in honeybees involves immersion in soapy water. This has been used due to its ability to allow water to fully enter the insect despite their outer coating. Freezing has been evaluated as a secondary step in the euthanasia process using a temperature of −18 °C and −80 °C and was found to not result in a humane death or required an extensive exposure period in certain species, such as G portentosa.

The objective of this study was to evaluate the efficacy and consistency of two 2-step euthanasia methods in 2 species of cockroaches, the CC and the MHC. The primary step was exposure to CO₂, a high concentration of isoflurane, or a combination of isoflurane and CO₂. This was followed by the use of either soapy water immersion or freezing (−80 °C). It was hypothesized that both soapy water immersion and freezing would be effective methods of euthanasia. While soapy water has not been scientifically validated, it has been used as a home-remedy insecticide and in euthanasia of honeybee colonies and would serve as an accessible alternative to facilities without a −80 °C freezer.

**Methods**

Approximately 800 CCs of various sexes and ages were used for this study. Cockroaches were added to 3.8-L bags in duplicate using their total weight in grams, rather than using the number of individuals, due to their size variation. Two bags were filled with 25 g of cockroaches, 2 with 50 g, 2 with 75 g, 2 with 100 g, and 2 with 150 g. To anesthetize the cockroaches, each bag had 100% CO₂ added at a rate of 4 L/min until the bag was filled.

Twenty-seven adult MHCs (16 males, 11 females) were divided into groups. Nineteen were exposed to 2 mL of isoflurane (USP; Piramal Critical Care) applied to a cotton ball and added to a 1-L plastic container containing 2–3 individuals for a total of 9 containers. An anesthetic agent applied to a cotton ball is an effective means of achieving anesthesia in invertebrate species as it allows for higher concentrations to be achieved. It is important to note that the cockroaches could come in contact with the soaked cotton ball. This anesthetic method, when used in Denver, CO, achieves a maximum concentration of about 37%. Additionally, 4 cockroaches were placed into two 3.8-L plastic bags, each with 2 individuals, and exposed to 100% CO₂ at 4 L/min until the bag was filled. Four cockroaches were placed into two 3.8-L plastic bags, each with 2 individuals, and exposed to 2 mL of isoflurane on a cotton ball followed by exposure to 100% CO₂ at 4 L/min until the bag was filled.

Induction time was recorded after the bag was filled. Induction was determined when there was no movement from any individual and there was no response to container disturbance. Adverse behaviors including perceived excitement associated with induction were also recorded. Each bag with CO₂ was left undisturbed for 15 minutes postinduction. Each container with isoflurane was left undisturbed for 30 minutes postinduction.

One bag from each weight group of CCs was placed in a −80 °C freezer for a period of 30 minutes, as were half of the MHCs anesthetized with CO₂, half of those anesthetized with isoflurane, and half of those anesthetized with the combination protocol. The bags and containers were monitored for movement at 2, 5, 10, 15, 20, and 30 minutes. The second bag of each group of CCs, and the other half of the MHCs, were submerged for 30 minutes in a 5% concentration of soapy water made using liquid dishwashing soap (Dawn Ultra Dishwashing Liquid; Procter & Gamble) and water. Submersion ensured their spiracles were not in contact with air. Based on the initial time of exposure to the secondary euthanasia method, individuals were monitored for the presence of life for a
period of 1 to 3 hours after removal. The presence of life was indicated by movement and response to stimuli. If movement was noted at any point during the monitoring phase for the freezing group, individuals were reanesthetized and placed in the soapy water. If movement was noted in the soapy water group, then the individual was held submerged for an additional 10 minutes to ensure the spikes were not in contact with air. All individuals were macerated at the end of the study to ensure complete destruction of all neural tissue. Denver Zoo’s Animal Welfare Committee approved this study.

Statistical analysis

Results, which mostly consisted of summary statistics, are reported as mean and SD. Failure rates are reported as percentages (number of individuals that survived the euthanasia treatment over the total number of individuals exposed to that treatment); confidence intervals are provided.

Results

Both CCs and MHCs were successfully anesthetized using CO2 and had mean induction times of 1.62 ± 0.4 minutes and < 1 minute, respectively. The 19 MHCs were successfully anesthetized using an isoflurane-soaked cotton ball and had a mean induction time of 14 ± 4.2 minutes. The 4 MHCs that were anesthetized with both isoflurane and CO2 had an average induction time of < 1 minute. Adverse behaviors associated with CO2 anesthesia included an excitement phase, which included hissing, limb twitching, and trembling that lasted for ≤30 seconds. Adverse behaviors associated with isoflurane anesthesia included an excitement phase, which included tremoring and limb twitching that lasted for an average of 4.6 minutes. Adverse behaviors noted with the combination of isoflurane and CO2 included tremoring, hissing, defecation, and regurgitation, which lasted for < 30 seconds. The combination of CO2 and isoflurane did not result in faster induction times or fewer complications compared to CO2 alone.

One CC was noted to have movement during the euthanasia process. The CC was in the 75-g soapy water group, and movement was noted after being submerged for 15 minutes. This individual was resubmerged for 10 minutes, and no movement was noted subsequently. Two female MHCs in the isoflurane –80°C euthanasia group were noted to have movement 2.5 hours after removal from the freezer. As stated previously, these MHCs were reanesthetized with isoflurane and exposed to soapy water. The CO2 plus soapy water method had a failure rate of 0.2% CI (−0.1% to 1.5%), and the isoflurane plus −80°C method had a failure rate of 0.5% CI (0.005% to 1.9%). The other groups had a failure rate of 0.0%.

Discussion

Euthanasia for cockroaches has been historically described as challenging. With the veterinary community advocating for more humane methods and consideration for providing a good death for all animal species, there is a need for better euthanasia methods in cockroaches and other invertebrates. The AVMA currently recommends mostly 2-step methods, involving CO2 or isoflurane anesthesia followed by a secondary method, such as freezing, immersion in alcohol or formaldehyde, or injection with drugs, such as pentobarbital. Injectable methods are not practical for mass euthanasia, which is often necessary in large collections. A recent study that assessed freezing, immersion in multiple solutions, and intracelomic injections in 4 cockroach species, as well as an isoflurane overdose, found the majority of methods approved by the AVMA were inconsistent or ineffective. For example, freezing MHCs at −18°C and −80°C was found to be 20% and 90% effective, respectively. This differs from the results of the current study, where freezing at −80°C had greater than a 99% (412 successful response to the treatment over 414 total exposed to that method) success rate across the 2 species evaluated. One plausible reason for the difference is that the cockroaches in this study remained exposed to the primary method while they were in the freezer, which likely minimized chances for arousal.

A novel technique in these species, namely, immersion using 5% soapy water, was also effective as a secondary euthanasia method for both species of cockroaches. This method offers an alternative euthanasia option for institutions or private establishments that may not have access to a −80°C freezer. Additionally, if further diagnostics, such as histopathology, are required, this method may be superior in preserving tissues, as freezing typically destroys tissue architecture and cell structure.

The 2-stage euthanasia methods evaluated in this study were found to be effective and consistent in both cockroach species. Both the soapy water and freezing methods may be simple and practical options for secondary rapid, large-scale euthanasia of cockroaches. Until these techniques receive broader use, however, the authors recommend following euthanasia with a final method, such as maceration, when possible, due to the 3 instances of failure.

In conclusion, the use of a 5% concentration of soapy water or freezing in a −80°C freezer was a successful secondary technique for euthanasia of MHCs and CCs after anesthesia with CO2, isoflurane, or a combination of isoflurane and CO2. There was no apparent benefit to using both CO2 and isoflurane for anesthesia induction. While these results are promising, alternative 2-step methods and the application of different concentrations of agents should be evaluated to provide additional euthanasia options.

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