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Research Article

Efficacy testing of Goodnature A24 self-resetting rat traps for wild house mice (*Mus musculus*)

Aaron B. Shiels[1,*], Danika R. Spock[1], Tyler Cochran[1] and Laurie Baeten[1,2]

[1]*USDA, APHIS, Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA*
[2]*U.S. National Park Service, Fort Collins, CO, USA*

**Corresponding author*
E-mail: *aaron.b.shiels@usda.gov*

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Abstract

House mice (*Mus musculus*) are worldwide pests in urban, agricultural, and natural settings. Goodnature® A24 rat+stoat self-resetting traps (A24s) are used globally for invasive rat control, yet adequate efficacy testing has not occurred against house mice. Our objective was to test efficacy of A24s against wild house mice. We first used cage/pen trials to determine whether the time from A24 impact to death was short and met international animal welfare standards (New Zealand National Animal Welfare Advisory Committee [NAWAC]). We also varied lure type (peanut butter vs. Goodnature chocolate lure) and trap configuration (vertical vs. angled in a trap stand) to assess trap attractiveness. Of the 80 mice tested, 67 triggered A24s, and just three required our intervention and euthanasia because they were still alive 2 minutes after being struck by the A24. Time to death for the remaining 64 mice averaged (± SE) 50.9 ± 2.6 seconds (median: 46.8 seconds, range: 19.8–120 seconds). Thus, A24s passed NAWAC standards of Class B for kill-traps against house mice. Although mice frequently contacted A24s during the 3-day trials, average time to trigger was 4.6 ± 0.6 hours and 13 mice never triggered A24s. Baiting A24s with peanut butter resulted in significantly greater mortality (98%) than by using Goodnature chocolate lure (70%). Mice triggered A24s baited with peanut butter 2.3 times faster than Goodnature chocolate lure, and 2.7 times faster if A24s were angled in trap stands rather than positioned vertically. In further trials, we released groups of up to five mice into a 24 m^2 arena containing either two A24s or two snap-traps+two A24s. Two A24s in the arena resulted in all 25 mice triggering A24s and dying; mice were undeterred from triggering A24s when mouse carcasses were near A24s. When snap-traps and A24s were present, one of 38 mice survived the 9-day trials. If snap-traps were reset each 24 hours they killed significantly more mice than A24s, yet if they were not reset during trials the A24s killed significantly more mice than snap-traps. A24s appear adequate for use against house mice, especially if baited with peanut butter and angled in trap stands.

Key words: free-ranging laboratory trials, humaneness testing, New Zealand National Animal Welfare Advisory Committee (NAWAC) standards, peanut butter bait/lure testing, vertebrate pest management, Victor® mouse traps, wild rodent control

Introduction

House mice (*Mus musculus* Linnaeus, 1758) are one of the world's most widespread invasive vertebrates, and they are known to damage property

and resources in urban, agricultural, and natural settings (Brown et al. 2004; Angel et al. 2009; St Clair 2011; Garba et al. 2014; Witmer and Shiels 2018). In urban settings within the U.S. and U.K., 36–49% of surveyed residents reported mice in their multi-family dwellings (Sked et al. 2021). House mice may carry and spread zoonotic diseases and can cause allergies in humans (Smith et al. 1993; Phipatanakul 2002). The presence and infestations of house mice have been reported to increase human stress and fear (Randler et al. 2012). These negative effects make house mouse elimination and population reduction a priority in the many areas where they are unwanted (Garba et al. 2014; Sked et al. 2021). The most common forms of house mouse control are by use of generalist rodenticide baits containing toxicants and the use of single-catch traps such as snap-traps (Witmer 2019).

Goodnature® A24 rat+stoat self-resetting traps (also referred to as A24s) are used extensively for invasive rat (*Rattus* spp.) control in a variety of states, countries, and environments (Carter et al. 2016; Shiels et al. 2019; Gronwald and Russell 2022; Baldwin et al. 2022, Crampton et al. 2022). Because A24s were developed for invasive rat and stoat control, A24s have not been formally tested against house mice. These traps represent an alternative to both toxic baiting programs that often require frequent rebaiting and elicit elevated environmental risk, and single-catch traps that commonly require high labor costs to maintain due to the frequent rebaiting and resetting of traps required for them to be effective (Witmer 2019). Unlike single-set snap-traps (e.g., Victor®) where animals access the bait and the trap along an uncovered surface, A24s consist of intersecting vertical and horizontal cylinders where rodents must scale the inside of the cylinder walls to reach the bait and trigger. The multi-kill component of the A24 trap is associated with a CO_2 cartridge that powers a piston directed at the head of a rat or stoat triggering the trap. Once impact occurs, the piston automatically self-resets. A24s can dispatch up to 24 rats with one CO_2 cartridge and it can maintain attractiveness and functionality for 4–6 months if used with a long-life lure (Goodnature chocolate lure) (Bogardus and Shiels 2020).

Although A24s could be an effective tool to control house mice, they should be tested for efficacy and humaneness prior to widespread use and landscape deployment. Because house mice are so much smaller than the target rats and stoats for which A24s were developed and tested to efficiently kill (e.g., house mice are > 5–20 times smaller than target wild rats in Hawaii; Shiels et al. 2020), it is plausible that the A24's piston may entirely miss the house mouse that triggers the A24 or it may maim the mouse. Although there are many interpretations of what constitutes a humane death, a short period from administration of the lethal action to death is a key characteristic outlined in animal welfare regulations, such as the New Zealand National Animal Welfare Advisory Committee (NAWAC).

Figure 1. The two A24 trap configurations tested: vertical (left image), and angled in trap stand (right image). Images from: http://www.goodnature.co/.

For kill traps to be acceptable under NAWAC guidelines (Class B Kill Traps, Appendix D, page 16), there are maximum allowable numbers of animals retaining corneal reflexes after 3 minutes and 5 minutes for Class B designation, and 30 seconds and 3 minutes for Class A designation (NAWAC 2019). For example, according to Class B guidelines, either 10 of 10 or 13 of 15 target animals must be rendered irreversibly unconscious within 3 minutes of capture (Morriss and Warburton 2014; NAWAC 2019). Higher sample sizes reduce the risk of an effective trap being rejected. For example, under the same Class B guidelines, 40 of 50 target animals must be rendered irreversibly unconscious within 3 minutes of capture to pass this animal welfare standard for kill traps (NAWAC 2019).

The objective of our study was to test the efficacy of A24 traps against wild house mice. Using controlled pen trials in the laboratory, we first determined whether the time from A24 impact to death was short and met international animal welfare standards of NAWAC Class B Kill Traps. We also varied lure type (peanut butter vs. Goodnature chocolate lure) and trap configuration (vertical vs. angled in a trap stand) to assess A24 trap attractiveness. Once these animal welfare standards were proven with our test subjects, we then used arena trials to simulate field conditions for further efficacy testing and to compare A24s to classical snap-traps. Our goal of these later trials was to determine which trap type (snap-traps or A24s) performed best under simulated field conditions with up to five wild mice present at once. We made the following predictions:

1) Given the small size of the house mouse and the A24 trap designed for larger mammals, we expected several triggered traps that resulted in misses and injuries.

2) A24s baited with peanut butter would be more attractive than Goodnature chocolate lure.

3) Angled A24s within a trap stand would help facilitate entry and triggering by mice relative to the vertical trap setting that is typically used for targeting rats (Figure 1).

4) During free-ranging conditions with multiple mice present in the arena, mice would be deterred from traps when a carcass was present next

to the trap, and therefore several survivors would result from each grouped trial after the first few mice were killed by A24s and snap-traps.

5) Snap-traps would be more effective than A24s because unlike A24s the bait is uncovered in snap-traps and does not require entry into a closed device to access the bait and trigger.

Materials and methods

Acquiring and maintaining house mice

House mice for this study were live-trapped using Sherman traps in livestock farms in Northern Colorado, and brought to the United States Department of Agriculture's (USDA's) National Wildlife Research Center (NWRC) to complete all trials. Upon arrival to the NWRC, mice were quarantined for 2 weeks and then each individual received a radio-frequency identification (RFID) tag (BioMark®, model MiniHPT10, 10 mm) that was inserted between the shoulder blades with an RFID tag applicator. To safely insert the RFIDs, mice were sedated using isoflurane. Mice were initially kept one individual per cage, in numbered plastic cages with wire lids. They were fed *ad libitum* a maintenance diet of Laboratory Rodent Diet pellets (www.labdiet.com) and water, and apple slices were added to each cage weekly. A den tube (10 cm × 4 cm; l × h) and corncob bedding were placed on the floor of the cages. All aspects of this project were approved under the USDA NWRC's Institutional Animal Care and Use Committee (IACUC) study protocol QA-2995. Mention of a company or commercial product does not mean endorsement by the U.S. government.

Pen trials

The first set of trials used small plastic pens (60 cm × 60 cm × 60 cm) to determine efficacy of A24s and to specifically test the ability of the A24 to kill house mice within the established animal welfare guidelines for Class B kill-traps (NAWAC 2019). Each pen had a food dish with rodent pellets, a plastic tube (10 cm × 4 cm; l × h) for denning and hiding, and wood shavings lined the floor (Figure 2). Five pens were run simultaneously with one mouse per pen. Each pen had a video camera for surveillance and a clear plastic (5-mm thick) screen partly covered the top of the pen to prevent mice from jumping from the top of the trap out of the pen (Figure 2). Mice were acclimated to their pen for 24 hours prior to placing an armed A24 into the pen. The room housing the pens had a light cycle set to 12 hours of light and 12 hours of darkness.

To determine if death by A24 met NAWAC animal welfare guidelines of whether 80% of mice triggering A24s were deemed irreversibly unconscious within 3 minutes of impact, we recorded the duration from A24 triggering to confirmation of death (i.e., irreversible unconsciousness was determined by using the palpebral (blinking) reflex). Additionally, our veterinarian (LB)

Figure 2. Photos of pen trial set ups with the A24 trap angled in the trap stand (left photo) and the vertical configuration of the A24 (right photo; a mouse is present for scale). All pens had a video camera for surveillance and a clear plastic screen that would partly cover the top of the pen to prevent mice from jumping from the top of the trap out of the pen. Also pictured are black plastic hides in each pen, and in the left photo a white food dish is visible in the upper left corner of the pen as well as a water tube extending through a hole in the middle of the far wall (left photo). Photos by Aaron B. Shiels.

conducted post-mortem examinations (i.e., necropsy) and noted the location of impact. Our experimental set up included the use of video cameras at each pen, and a computer monitor in the adjacent room where staff would watch the first hour following adding A24s to the pen in real time, and afterwards we relied on an audio receiver-speaker system (vtech® Enhanced Range Digital Audio Monitor, model DM1111, baby monitors) to alert staff that the A24 had been triggered. A24s make a loud sound when triggered. Once the staff had observed or heard an A24 firing, they immediately started a stopwatch that was then stopped once death was confirmed using the palpebral reflex method. If 2 minutes had passed since impact and the mouse was still alive, it was immediately euthanized with CO_2 gas. Although we realize euthanizing after 2 minutes is a slight deviation from the NAWAC method of waiting for 3–5 minutes by Class B kill-trap guidelines, we chose to be more conservative and disallow impacted mice to live beyond 2 minutes.

There were 80 adult mice (40 male, 40 female) used in the pen trials, and the size ranges were 16.0–30.9 g (mean ± SE: 22.5 ± 0.4 g, median: 22.4 g). Our methodology allowed us to combine the NAWAC testing with assessing trap efficacy under different A24 trap variables. The two main variables tested to assess A24 attractiveness to mice were: lure type (Skippy® peanut butter vs. Goodnature chocolate lure) and trap configuration (angled in a trap stand vs. vertical; Figure 1). The lure was placed in excess in the Goodnature lure basket, which attaches within the trap just above the trigger. For the vertical A24 configuration, we fastened the A24 to a wood plank, and used heavy duty tape to secure it to the pen wall (Figure 2). There were 20 mice of equal sex used for each lure type × trap configuration treatment. To account for house mice potentially being more active at night

Figure 3. Photos of the arena used to test A24 traps against multiple free-ranging house mice and to compare relative attractiveness of A24 traps vs. mouse snap-traps. Left photo shows the full arena (24 m^2) with a video camera above each corner of the arena used to monitor traps for mouse visits. Right photo shows A24 trap on a trap stand in upper left, black plastic hides in foreground and background, black den box (large) at center-right, and a food and water station (clear plastic) in lower right. Photos by Aaron B. Shiels.

than day, we acclimated half of the house mice (i.e., n = 40) to be on an opposite day/night cycle so we could simulate nighttime during the working hours. We determined that there were equal frequencies of mortality between mice on day/night cycle versus mice on a night/day cycle (χ^2 = 0.12, df = 1, p = 0.7301).

Each trial in the study lasted 4 days; the first day was an acclimation period to the pen without an A24 present, followed by 3 consecutive days of trial with an A24 present and armed for 7 hours each day. Thus, A24s were only present and active in the cages for 7 hours each day, which was the period that NWRC staff were present. We randomized the order of treatments but Goodnature chocolate lure was trialed first with angled and vertical trap orientation, and then peanut butter was trialed with angled and vertical trap orientation. No mice were re-used for any of the trials. Mice were provided water and a PVC hide for shelter during the trial period, and food was only present for part of the trial period. Further details of the 3-day trial were:

Day 1—food (rodent diet pellets) present with A24;
Day 2—food had been withheld 16 hours prior to Day 2, and food remained absent while the A24 was present (until Day 2 ended);
Day 3—food was present with the A24. Trial ended this day unless the mouse was killed in days prior.

Arena trials with two A24s

Once A24s were deemed humane and in compliance with international standards of animal welfare for use on house mice (i.e., pen trials above), we tested the A24s in more realistic conditions of a 4.9 m × 4.9 m (24 m^2) arena (Figure 3). The arena is made of the same plastic sheeting as used in the pens described above. Arena trials had woodchip bedding, two food

and water stations with RFID tag readers (BioMark®, 18 cm inner-diameter Terrestrial Antennas attached by cable to a model SM303 Antenna Multiplexer and a RM310 Data Logger BLE) beneath them, and 20 hides (4 den boxes [28 cm × 28 cm × 16.5 cm; l × w × h; with two 5.4 cm diameter entry-exit holes], 4 hide boxes [13 cm × 13 cm × 6.5 cm; with two 3 cm diameter entry-exit holes], 4 "half pipes" [PVC pipes cut in half; 15 cm × 9.5 cm × 6 cm], and 8 small tubes [10 cm × 4 cm; l × h]). The arena had a light cycle set to 12 hours of light (during staff working hours), and 12 hours of darkness. Constant temperature (20–22 °C) and humidity (40%) were maintained throughout all the trials except that the last 10 individuals in the "two snap-trap+two A24 trials" (i.e., final two trials; see below) had air temperature set to 29.5 °C and 50% humidity. Mice were allowed to acclimate to the arena without traps present for 3 days prior to a trial beginning. Trials (with traps present) lasted up to 9 days. Each arena trial with two A24s present had five house mice admitted into the arena simultaneously, and there were five trials conducted (n = 25 mice in total) with average mouse weight of 24.5 ± 1.0 g (median = 25.0 g, range: 12.6–32.3 g). The two A24s were positioned at opposite ends of the arena and about 1 m from the arena walls. None of the mice from pen trials were reused in these trials. Unlike the pen trials, traps remained in the arena continuously for the duration of each trial. Additionally, to further encourage mice to visit traps, food was withheld at 08:00 on day 3 (i.e., 48 hours after traps were placed in the arena) for 48 hours (i.e., food returned into arena on day 5 at 08:00); food was again withheld on day 8 (removed at 08:00) and the trial ended on day 9 at 16:00. Based on the results of the earlier pen trials, the two A24s placed in the arena were baited with Skippy peanut butter and had the angled orientation supported by the Goodnature trap stand (Figures 1, 3). Mouse carcasses resulting from death by A24s were left in place for approximately 24 hours before removal and disposal; we aimed to remove them after 24 hours but if the removal period occurred outside staff working hours the carcasses remained slightly longer (up to 40 hours) until staff returned to work. Video cameras were established to record mouse interactions at both A24s, and NWRC staff could generally view the incidences of A24 triggerings at < 24 hour intervals. We recorded the time from the start of the trial to each individual's triggering event, and we noted the A24 that the mouse triggered and whether or not a carcass was near the A24 that was triggered or at the other A24 in the arena that was not triggered.

Arena trials with two snap-traps+two A24s

The final arena trials followed the same design as the arena trials with two A24s, with the exception that two Victor mouse snap-traps were added, such that four traps in total (two snap-traps and two A24s, each baited with peanut butter) were present near each of the four corners of the arena. These trials were set up to test if one trap type was more attractive to house

mice than the other. There were four or five mice added at once to the arena and allowed to acclimate for three days prior to adding the snap-traps and A24s. Because snap-traps are single-set traps, we tested the efficacy of traps when snap-traps were not reset during the duration of each trial (n = 3 trials), and when snap-traps were reset each 24 hours if they had been triggered or the bait had been removed (n = 5 trials). These trials were to mimic field operations of trappers' frequency of returning to service snap-traps. Like our previous arena trials, we left mouse carcasses next to traps (or in snap-traps during the 'not resetting' treatment) for 24 hours. Trials lasted up to 9 days. A total of 39 mice were used yet seven of the mice from the pen trials were reused in these trials. Using video surveillance, we recorded the time from onset of the trial to each individual's triggering event and the type of trap triggered during both resetting and not resetting the snap-traps.

Statistical analysis

For the pen trials, the frequency of A24 mortality between lure type (peanut butter vs. Goodnature chocolate lure) and trap configuration (angled vs. vertical) were analyzed with separate chi-square tests. The sex of the mouse was also tested by chi-square to determine if it influenced the frequency of mortality. We used a 2-way ANOVA (on log-transformed data to meet assumptions of normality and equal variances) to compare lure type and trap configuration, and their interaction, for the amount of time from the trial's beginning to each individual's triggering of the A24.

For the arena trials, we used chi-square tests to determine if mice were more or less likely to go into an A24 if a dead carcass was near the A24, or into a snap-trap if a dead carcass was near the snap-trap. For the snap-trap+A24 trials, we used chi-square analysis to compare the frequencies of mortalities caused by snap-traps versus A24s when resetting and not resetting snap-traps was practiced. We also compared the frequency of the first kill for each trial between snap-traps and A24s. All statistical analyses were conducted in R (version 3.4.1) and significance was based on $p < 0.05$.

Results

Pen trials

During the 3-day trials, 67 of the 80 trialed mice were killed by A24s (i.e., 84%). There was just one of the 13 surviving mice from the peanut butter baited A24s and the remaining 12 mice were from the trials where Goodnature chocolate lure was offered in the A24s. There were three mice (all in the peanut butter baited A24s set in vertical trap orientation) that had to be euthanized after being struck by the A24 because they were still alive after 2 minutes following triggering the A24, and 2 minutes was our *a priori* cutoff to intervene and euthanize a mouse struck by the trap. These three mice (two females, one male) were the 41st, 42nd, and 47th mouse that

Table 1. Results of house mouse trials with A24 traps baited with either peanut butter or Goodnature chocolate lure. Mice trialed that triggered traps included n = 39 for peanut butter (19 vertical, 20 angled), and n = 28 for Goodnature chocolate lure (15 vertical, 13 angled).

Measurement	Peanut butter	Goodnature chocolate
Mortality (%)	97.5	70.0
Mortality by sex ratio (F:M [F/M])	19:20 [0.95]	13:15 [0.87]
Time between triggering & death (sec)	62.1 ± 10.2	57.3 ± 3.5
Trap exposure time until triggering (hours)	2.97 ± 0.68	6.84 ± 1.09
Mortality on first day of trap exposure (%)	84.6	57.1

Table 2. Results of house mouse trials with A24 traps positioned either vertical or angled within a trap stand. Mice trialed that triggered traps included n = 34 with traps in vertical position (19 peanut butter, 15 Goodnature chocolate lure), and n = 33 for traps in angled position (20 peanut butter, 13 Goodnature chocolate lure).

Measurement	Vertical	Angled with ramp
Mortality (%)	85.0	82.5
Mortality by sex ratio (F:M [F/M])	15:19 [0.79]	17:16 [1.1]
Time between triggering & death (sec)	71.6 ± 11.4	48.2 ± 3.0
Trap exposure time until triggering (hours)	6.63 ± 0.97	2.47 ± 0.68
Mortality on first day of trap exposure (%)	58.8	57.1

triggered A24s in our trials, and they had the following fatal (or near fatal) injuries from the A24 trap: pulmonary hemorrhage, puncture to the right of the sternum, and skull fracture. Based upon necropsies of the 64 mice (i.e., 96%) that died from triggering the A24, 61 died from spine or skull fracture (crush) and three died from an internal hemorrhage. Thus, A24s passed NAWAC standards of Class B for kill-traps against house mice. All 64 killed by A24s experienced what we consider (and in accordance with international standards of NAWAC 2019) to be an instant death, where death was confirmed within approximately 1 minute of A24 impact (mean ± SE: 50.9 ± 2.6 seconds, median: 46.8 seconds, range: 19.8–120 seconds). Note that death probably occurred earlier than recorded in most cases. This discrepancy is due to our methodology and trial set up whereby we observed the A24 impact (using a video camera or audio monitoring system) and started timing from an adjacent room. It took several seconds for the observer to enter the room and confirm death by palpebral (blinking) reflex. The affected mouse was almost always dead upon first attempt of palpebral reflex check. It took surprisingly long for mice to trigger A24s during the pen trials, as the average time for the 67 mice to trigger an A24 was 4.6 ± 0.6 hours (median: 2.1 hours, range: 1 minute–20.4 hours).

When treatments (i.e., lure type, and angled vs. vertical trap configuration) were compared for mouse mortality, A24s baited with peanut butter resulted in significantly more mortalities (i.e., 98%) than A24s baited with Goodnature chocolate lure (χ^2 = 11.57, df = 1, p = 0.0021; Table 1). Despite each mouse frequently interacting with the A24s, including jumping and climbing on exterior and interior portions of the trap, post, and ramp, there were 13 mice (i.e., 30%) that did not trigger the trap within the 3 days of exposure to the A24. There was no significant difference in the frequency of mortalities between A24s placed vertically and A24s angled in the ramp (trap stand) (χ^2 = 0.07, df = 1, p = 0.7903; Table 2). The sex of the mouse did not influence

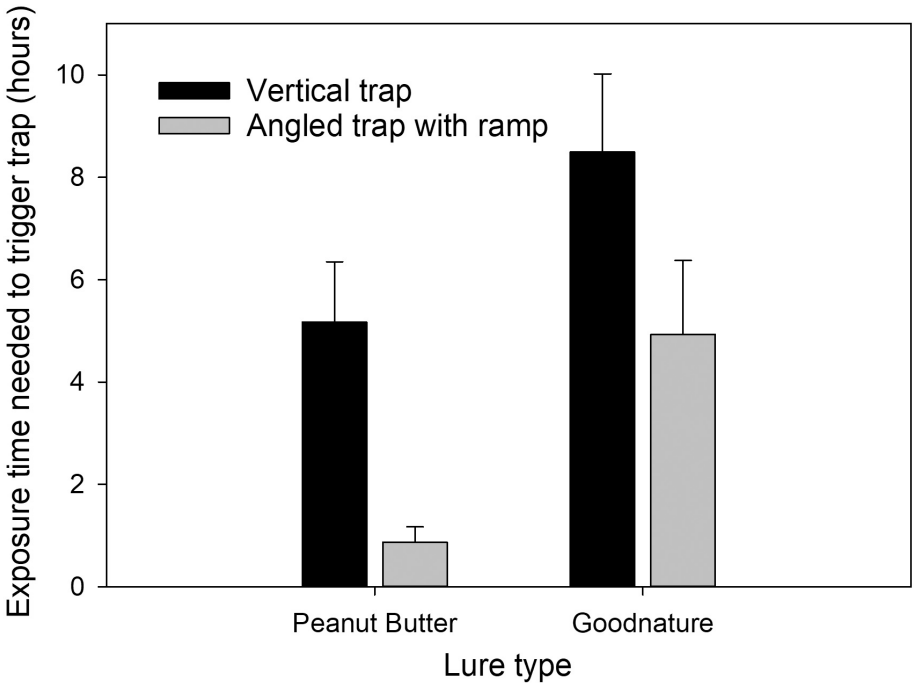

Figure 4. Mean ± SE time (hours) that individual house mice were exposed to a baited and armed A24 trap in their cage before triggering the trap when peanut butter or Goodnature chocolate lure was used as the bait/lure, and when the configuration of the trap was either vertical or angled with a trap stand. Mice trialed that triggered traps included $n = 39$ for peanut butter (19 vertical, 20 angled), and $n = 28$ for Goodnature chocolate lure (15 vertical, 13 angled).

the frequency of mortality ($\chi^2 < 0.01$, df = 1, $p > 0.99$ for both treatments), as near-equal ratios of females and males triggered and died from A24s (Tables 1, 2).

When treatments were compared for the amount of time a mouse was exposed to the trap prior to triggering the trap, which we considered as a measurement of relative attractiveness of the trap to mice, there were significant differences in lure type ($F_{1,36} = 13.66$, $p = 0.00046$; Table 1) and trap configuration ($F_{1,36} = 17.80$, $p = 0.00008$; Table 2), but there was no significant interaction between these two treatments ($F_{1,36} = 0.84$, $p = 0.36364$; Figure 4). Mice triggered traps baited with peanut butter 2.3 times faster than those baited with Goodnature chocolate lure (Table 1). Although approximately 85% of mice triggered the A24 and died on the first day when the A24s were baited with peanut butter, only about half of the mice triggered the traps and died on the first day while using Goodnature chocolate lure and the remaining mice triggered traps on day 2 (when food had been withheld) and day 3. Mice triggered traps placed in the angled configurations with the ramp 2.7 times faster than those traps placed vertically (Table 2). Just over half of the mice triggered the traps and died on the first day with angled or vertical trap configurations. We were surprised that it took so long for mice to trigger traps (averaging 4.6 hours across all trials), especially when there were few options for exploration in the pens during these trials, yet baiting A24s with peanut butter and using the angled trap configuration with ramp resulted in the quickest triggering of A24s by mice (i.e., average of 0.9 hours, Figure 4).

Arena trials with two A24s

When trials of five mice at once were free-ranging in the 24 m^2 arena and had continuous access to two A24s baited with peanut butter and angled (in trap stand), all mice trialed (n = 25) died from triggering A24s. Within 48 hours of placing A24s in the arena for each of the five trials, and thus prior to withholding any food, 68% of mice died by triggering A24s (Day 1: 32%, Day 2: 36%, Day 3: 20%, Day 4: 8%, and Day 8: 4%). Although four of the five trials ended with the final mouse being killed by an A24 on the fourth day, there was one trial where a mouse survived until the 8th day before triggering the A24. This final mouse was killed by an A24 approximately 14 hours after withholding food for the second time during the trial. There were no occasions where mice triggered the A24 and survived. Once the A24s were placed in the arena, the time it took for a mouse to trigger an A24 was 41.9 ± 7.6 hours (mean ± SE), and the average time it took for the first mouse in a group of five to trigger an A24 was 17.1 ± 6.0 hours (range: 4.6–39.9 hours).

While determining whether a mouse was more or less likely to trigger an A24 when there was an existing dead mouse next to the trap, we recorded nine mice that had triggered A24s despite a carcass next to the same trap at the time of triggering. Those nine mice were statistically compared to the nine mice that had the opportunity to choose an A24 with a mouse carcass next to it but instead chose the other A24 (with carcass absent) in the arena to trigger. There was no significant difference in the frequency for which mice triggered A24s with a carcass present or absent ($\chi^2 < 0.01$, df = 1, $p > 0.99$).

Arena trials with two snap-traps+two A24s

When trials with four or five mice at once were free-ranging in the 24 m^2 arena with continuous access to two snap-traps and two A24s (n = 38 mice total, 8 trials total), all mice triggered one of the trap types and died except for one individual that had to be euthanized due to an injury from a snap-trap, one individual that died of natural causes in the arena, and one individual that survived the trial duration (9 days). Of the eight trials conducted, three trials did not have snap-traps reset or rebaited when the snap-trap was triggered, and five trials were executed with snap-traps being reset and rebaited at each 24 hour servicing event. When all eight sets of trials were considered, there were 15 mice killed by A24s and 20 mice killed by snap-traps, and the frequency of mouse kills by trap type was not significantly different ($\chi^2 = 0.91$, df = 1, $p = 0.339$). However, when the five trials where snap-traps were reset were analyzed, there was a greater frequency of mice killed by snap-traps (n = 17) than by A24s (n = 6) ($\chi^2 = 8.05$, df = 1, $p = 0.0045$; Figure 5). When snap-traps were not reset when accidently triggered or when they killed a mouse, A24s killed significantly more mice (n = 9) than did snap-traps (n = 3) ($\chi^2 = 4.17$, df = 1, $p = 0.0412$;

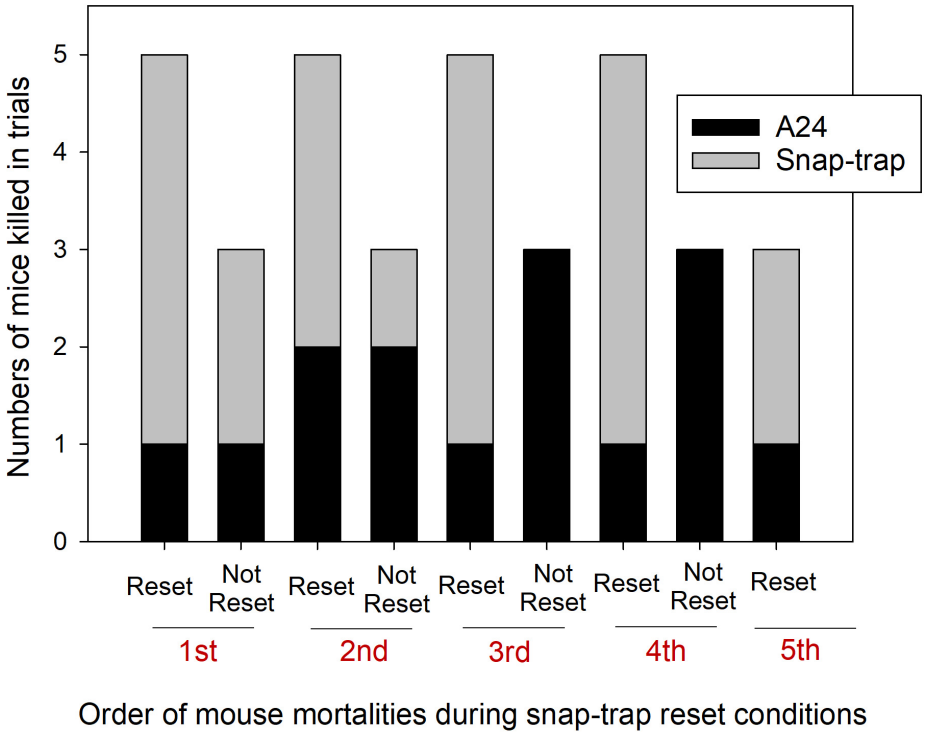

Figure 5. Results of arena trials where free-ranging house mice were simultaneously exposed to four traps (2 snap traps, 2 A24s) in the 24 m^2 space, and the order of mortalities shown (1^{st}–5^{th}, in red font) is during snap-trap reset conditions of either resetting snap-traps or not resetting snap-traps. There were five trials where snap-traps were reset each 24 hours, and three trials where snap-traps were not reset for the duration of each trial. Each trial had four or five mice, each trial lasted up to 9 days, and there were no mice remaining after the 4^{th} mouse died in the "not reset" treatment. Snap-traps were responsible for significantly more mortalities in the "reset" treatment, and A24s were responsible for significantly more mortalities in the "not reset" treatment (see Results).

Figure 5). There was a total of seven snap-traps that were triggered without successfully trapping or killing a mouse (two triggerings from the three trials where snap-traps were not reset if triggering occurred, and five triggerings from the five trials where snap-traps were reset as needed each 24 hours). On one of the trials where snap-traps were not to be reset if triggered, one snap-trap had bait removed the first night without triggering the trap and the trap did not attract or trap a mouse for the duration of the trial, and the other snap-trap was triggered the first night and wounded the mouse (later euthanized); therefore, all four remaining mice were eventually killed by A24s. There were no incidences where A24s fired and missed killing a mouse. Video surveillance showed that every mouse that was killed by a snap-trap (i.e., n = 20 mice) took approximately 30–120 seconds (mean ± SE: 51.0 ± 7.3 seconds; median: 30 seconds) to stop moving once the bar of the snap-trap impacted the mouse. Just one of the 15 mice killed by A24s was observed moving after being impacted by triggering the A24, and it was moving for about 30 seconds after the trap's impact.

As another method to assess the attractiveness of snap-traps versus A24s, the first trap resulting in a dead mouse was noted for each of the eight trials. Although just two of the eight trials had the first mouse killed

by an A24 (thus six of eight were killed by a snap-trap), there was not a statistically significant difference in frequency of first mouse killed between snap-traps and A24s (χ^2 = 2.25, df = 1, p = 0.1336). Similar to the trials where just two traps (both A24s) were active in the arena, the majority of mice killed among all eight trials with four traps each (2 snap-trap+2 A24s) occurred during the first 48 hours (i.e., 83%), which was prior to withholding any food. The day-by-day mortalities were: Day 1: 46%, Day 2: 37%, Day 3: 9%, Day 4: 9%. Although seven of the eight trials ended with the final mouse being killed by a trap by the fourth day, there was one trial (snap-traps reset) where a male mouse survived until the 9th day, which was also when the trial ended, and included two bouts of withholding food. There were no occasions where mice triggered the A24 and survived. Once the four traps were placed in the arena, the average time it took for a mouse to trigger a trap was 32.1 ± 3.8 hours (mean ± SE), and the average time it took for the first mouse in a group of five to trigger a trap was 8.5 ± 1.7 hours (range: 0.8–15.1 hours), which was approximately twice as fast as only having two traps (both A24s) in the same 24 m^2 environment with equivalent mouse density (see above section).

Similar to the arena trials with only two A24s present, it did not appear to affect mouse trapping to have mouse carcasses next to the snap-traps. While determining whether a mouse was more or less likely to trigger a snap-trap when an existing dead mouse was present next to the trap, we recorded seven mice caught in a snap-trap despite a carcass right next to the same trap at the time of triggering. Those seven mice were statistically compared to the seven mice that had the opportunity to choose a snap-trap with a mouse carcass next to it but instead chose the other snap-trap (with carcass absent) in the arena to trigger. There was no significant difference in the frequency for which mice triggered snap-traps with a carcass present or absent (χ^2 < 0.01, df = 1, p > 0.99). When A24s were examined, there were two mice killed in an A24 with a carcass next to the same trap, and three mice killed in an A24 that had the opportunity to choose an A24 with a mouse carcass next to it but instead chose the other A24 (with carcass absent). There was no significant difference in the frequency for which mice triggered A24s with a carcass present or absent (χ^2 < 0.01, df = 1, p > 0.99). There was no evidence of any mice feeding on mouse carcasses during any of our trials.

Discussion

Our findings demonstrate that when house mice trigger A24s, they experience an instant death that occurs within approximately 51 seconds. Unlike our prediction that the small sizes of house mice relative to rats would result in misses or injuries when the A24s were triggered by mice, there were few misses and injuries from A24 triggerings. The short time of

death from the A24, and the spinal or skull fracture that most frequently occurred from house mice triggering A24s, meets international animal welfare standards (NAWAC Class B for kill-traps). Although we were surprised how long it took mice to enter A24s in our small pen trials (average of 4.6 hours, median 2.1 hours) where there were limited objects to interact with aside from the A24s, this amount of time would not be considered unreasonable during field conditions when trappers generally check live- or snap-traps at a frequency of no less than each 24 hours. Unexpectedly, the presence of a mouse carcass next to a trap (snap-trap or A24) did not affect whether or not a mouse triggered the trap. Our prediction that snap-traps would be more effective (resulting in more kills) than A24s was only supported if the snap-traps were reset each 24 hours; otherwise A24s were more effective than snap-traps. Our pen and arena trials demonstrated that most mice were killed within 48 hours of placing the A24s in with the mice. Our lure and trap configuration treatments further demonstrated how A24s could be more attractive to wild house mice, and the use of peanut butter as bait rather than Goodnature chocolate lure significantly improved the time it took for mice to trigger A24s, as did placing the A24s in trap stands so they were angled rather than securing the A24s vertically. These subtle improvements to trap attractiveness that we uncovered, as well as the short time to death for mice when they trigger A24s, should provide users with a reliable alternative or supplement to other house mouse control tools.

In addition to the animal welfare trials that were performed on target rats and stoats as the A24 trap was developed (R Van dam, Goodnature, *pers. comm.*), there have been two other trials assessing A24s for animal welfare that occurred in the U.K.—one was with *R. norvegicus* (Berkenhout, 1769), and the other was with the wood mouse *Apodemus sylvaticus* (Linnaeus, 1758). These trials determined that the A24 trap meets criteria for humaneness for these targeted species. In a report provided by R. Van dam (Goodnature), the wood mouse trials resulted in 10 of 10 mice rendered irreversibly unconscious by lack of eye reflex in < 30 seconds since being impacted by A24s. The wood mice used in their study were approximately the same size (mean ± SE: 24.3 ± 1.9 g, range: 15.9–27.7 g) as the house mice that we trialed (22.5 ± 0.4 g, range: 16.0–30.9 g). The A24s were positioned 5 cm above ground for the wood mice, which was lower than our vertically positioned A24s at 12 cm above ground. Whereas other trials, including the previously described trial for wood mice, may have demonstrated class A for kill-traps (NAWAC 2019) on their species, we were not able to conclude for the house mouse the shorter time of death that would constitute class A partly or wholly due to our methodology. In our study, death probably occurred earlier than recorded in most cases because we were observing A24 impacts on a video monitor or using an audio system in an adjacent room to where the mice were on trial. Thus, there were several seconds of travel time to get into the adjacent room

from when we saw or heard the mouse triggering the A24 and for us to confirm death by lack of eye reflex. Using the described methodology, we only had 12 mice (i.e., 18% of those trialed) with time to death recorded ≤ 30 seconds, and for NAWAC Class A designation at least 80% of our trialed mice would have needed to have death confirmed within 30 seconds of A24 impact.

Baiting A24s with peanut butter improved A24 attractiveness over using Goodnature chocolate lure. Peanut butter baited A24s resulted in a 2.3 times reduction in the time for mice to trigger A24s, and it significantly improved mortality (98% mortality with peanut butter vs. 70% mortality with Goodnature chocolate lure). Peanut butter is perhaps the most common bait that is used worldwide during invasive rat and mouse trapping (King et al. 2011; Morriss and Warburton 2014; Shiels et al. 2019), and peanut butter is a standard bait used to meet or improve attractiveness to rodents when developing artificial lures (Jackson et al. 2016). Goodnature offers a lure basket that fits into the A24 and can be filled with a lure of choice, and it was these lure baskets that we used in our trials. Another alternative lure dispenser that is offered by Goodnature for the A24s is the automatic lure pump (ALP), which constantly squeezes lure from a pouch. The fresh lure drips through the trap entry and onto the ground, thereby ensuring that fresh lure is always present for 4–6 months. ALPs are commonly used for rat trapping in New Zealand (Gronwald and Russell 2022) and they have improved trap efficacy in Hawaiian forests where slugs and other organisms spoil the static bait placed in lure baskets (Bogardus and Shiels 2020). One important issue given the findings from our mouse trials is that Goodnature only offers the ALP with Goodnature chocolate lure, so an automatic squeezing mechanism of fresh peanut butter as a lure for the A24 does not exist. However, Goodnature has recently begun to market a "nut butter ALP". Given our positive results with peanut butter, the nut butter ALP may deserve future testing for its attractiveness to house mice.

The orientation of the A24, specifically whether it was secured vertically or angled, affected the time it took mice to trigger the A24. On average, mice took 2.7 times longer to trigger A24s when they were positioned vertically than if they were angled in a trap stand. For rat trapping, the vertical orientation (and securing the A24 12 cm above ground) reflects the manufacturer's instructions and is most used in the published literature as the traps are generally fastened to trees or posts (Carter et al. 2016; Shiels et al. 2019; Bogardus and Shiels 2020; Gronwald and Russell 2022; Baldwin et al. 2022; Crampton et al. 2022). Goodnature markets the trap stand as an alternative way to secure A24s for use indoors and outdoors, when trees and posts are not available, and when the traps are already primed and need to be moved around to target rodent hot spots. Although no published comparisons are available for vertical vs. angled trap orientation for rats or mice, Campbell and Hartley (2018) conducted laboratory trials attempting

to exclude non-target hedgehogs (*Erinaceus europaeus*, Linnaeus, 1758) from the A24s, and varying trap angle was part of their study design. In their trials, A24s secured in a Goodnature trap stands had the greatest potential risk because the hedgehogs placed their heads near the trigger of the unarmed A24 when it was angled in the trap stand but not when the A24 was placed vertically. Although findings from our study favor the A24 secured in a trap stand for eliciting house mouse trapping rather than placing the A24 vertically, there may be a downside to using the trap stand because it may be more encouraging toward trapping non-target species relative to a vertically positioned A24 (as reported in the hedgehog study). Assessing risk to non-targets is recommended for any trapping or pest control initiative. While Crampton et al. (2022) and Shiels et al. (*in press*) have tested efficacy of attaching excluder devices to A24s to ensure target rats visit and trigger A24s and non-target birds are excluded, such trials with mice would need to occur to ensure A24 efficacy is not reduced when mice are presented with excluders attached to A24s in the vertical and angled (trap stand) configurations. Attaching Goodnature plastic excluders at 0–2 cm above ground to vertically positioned A24s (as suggested in Crampton et al. (2022), and Shiels et al. (*in press*)) may encourage house mouse entry into A24s because of the proximity to the ground preventing a need for mice to jump up into an A24. Positioning the A24 at 2 cm above ground, without an excluder, may have the same faciliatory effect as positioning the excluder at 2 cm height. Again, these height and excluder variations would have to be tested with house mice before definitive conclusions and recommended uses could be made.

Utilizing our 24 m^2 mouse arena allowed for more realistic trials of A24 efficacy than the small pen trials, as groups of up to five mice were allowed to range freely with a multitude of hides, continuously available water, and excess food of their standard diet (rodent diet pellets) available for all but 80 hours during the 9-day trials. When two A24 traps were placed in the arena and five sets of trials with five mice grouped in each trial, all 25 mice were killed by the A24s within 8 days. Although the A24 trap density and artificiality of several aspects of the arena do not mimic most field settings, these trials were encouraging for demonstrating how high levels of house mouse control in closed populations can occur by using A24s. Although we were initially concerned that mice would observe others getting killed by traps and therefore would refrain from entering or triggering the A24s (or snap-traps), this behavior was not observed. Similar to our findings that mice were undeterred from triggering A24s that had a carcass from a previous kill next to the trap, A24 trappers in both New Zealand (Carter et al. 2016) and Hawaii (Kreuser et al. 2022; Crampton et al. 2022) have observed target rat carcasses directly beneath the A24s and their presence did not deter subsequent rats from visiting and triggering the A24. Additionally, visitation to A24s when rodent carcasses are present does not appear to be

influenced by using peanut butter bait versus Goodnature chocolate lure, as our study and that of by Carter et al. (2016) used peanut butter, whereas the Hawaii studies (Kreuser et al. 2022; Crampton et al. 2022) used Goodnature chocolate lure (in ALPs).

Our mouse trials in the arena with snap-traps and A24s demonstrated the importance of trap-servicing frequency. Whereas A24s can remain functional for 4–6 months when deployed with the ALP (Bogardus and Shiels 2020), snap-traps are single set and single catch traps. Our trials revealed that if snap-traps were reset each 24 hours then they killed significantly more mice than A24s, yet if they were not reset during trials then the A24s killed significantly more mice than snap-traps. Of note is that there were seven "misses" with snap-traps, where the trap was sprung without catching a mouse. One of these misses resulted in an injury that required euthanizing the mouse. Morriss and Warburton (2014) have tested modifications to rat snap-traps that better direct the target species to the trap, increasing the efficacy of rat snap-traps. Such modifications could be applied to mouse snap-traps to potentially increase efficacy and animal welfare. We found that all 20 mice killed by snap-traps in our study took approximately 30–120 seconds (mean ± SE: 51.0 ± 7.3 seconds; median: 30 seconds) to stop moving once the bar of the snap-trap impacted the mouse. Because we were not observing these triggerings real-time, but we instead re-watched video footage recorded from the previous day, we were not able to follow NAWAC guidelines (NAWAC 2019) of immediately checking mice for irreversible unconsciousness to assess whether snap-trapped mice met the animal welfare guidelines like those met for A24s during our A24 pen trials.

There are situations where frequent resetting of snap-traps is practiced when attempting to control house mice, such as inside and outside structures in residential or urban settings. In such settings and when personnel are available to check and reset snap-traps regularly, use of mouse snap-traps may be a favored trap over A24s to maximize rapid mouse control. In more isolated settings, such as in natural areas, or when it is unfeasible for personnel to frequently reset snap-traps, the use of A24s to control mice may be more efficient. Although the cost of an individual A24 (> $150 each) is many times greater than the cost of individual mouse snap-traps ($1 each), the long rebaiting and servicing intervals associated with A24s should greatly reduce personnel time required to visit and service A24s vs. single-set traps like snap-traps (Bogardus and Shiels 2020). Rodent control operations often use multiple tools and use of snap-trapping with A24s could be a viable strategy for optimizing rodent control. In Hawaii, rat snap-traps (baited with peanut butter) were used simultaneously with A24s (with ALPs filled with chocolate lure) in attempt to control invasive rats and mice in a remote segment of native forest where traps could only be serviced on 3-month intervals (Shiels et al. 2019). In that study, black rat (*R. rattus* Linnaeus, 1758) populations were reduced by the dual trap types,

but the house mouse population was not reduced. There are several possible explanations for the failure of rat snap-traps and A24s to reduce house mice in the field, including competitive dominance of black rats over house mice (Shiels et al. 2013) that may have precluded mice from gaining access to armed and baited traps. It was encouraging to discover that within our lab setting at NWRC house mice were active during the dark and light, and if that behavior transfers to the field then there may be some possibility of day/night niche partitioning between nocturnal rats and semi-diurnal house mice that would allow these rodents to visit A24s despite competitive dominance. Additional concerns noted within the Shiels et al. (2019) study are that rat snap-traps were found to work poorly against house mice (Shiels et al. 2017), and if house mice had been the main target rodent in the rodent control project then mouse snap-traps should have been used, as successfully shown in grasslands in Hawaii (Shiels et al. 2017; Liang et al. 2022). Based on our laboratory study, two additional factors that may have contributed to the low efficacy of A24s against house mice in Shiels et al. (2019) were the use of chocolate lure in the ALPs (rather than peanut butter) and possibly the vertical positioning of the A24s. However, in accordance with our study, the vertical positioning of A24s should have only extended the time that mice needed to trigger A24s (i.e., trap exposure time) over that of the angled trap stand configuration and not altered the mouse mortality.

Finally, the one surviving mouse out of 38 mice trialed in the arena with snap-traps and A24s underscores the importance of individuality of behavior in rodent populations, which further highlights the possibility that not all individuals in a population will visit a control device (e.g., bait station or trap) or be trapped even when multiple trap types are used simultaneously, and trap densities are relatively high. This is an important factor when planning rodent control projects where the goal is to drive the mouse or rat population down as close as possible to zero individuals, or by ensuring zero individuals remain when rodent eradication is the goal (Samaniego-Herrera et al. 2018). Some evidence exists for the success of A24 trap use being largely affected by target rodent density. For example, Warburton and Gormley (2015) concluded that single-capture traps were more efficient to use when target vertebrate pests are at low densities, whereas at higher densities fewer multiple capture traps were more efficient.

Conclusions

A24s appear adequate for use against house mice, as demonstrated in both individual pens and larger, free-ranging settings with multiple mice and trap options. A24s met international standards of animal welfare by resulting in quick deaths once house mice trigger A24s. The higher attractiveness of house mice to peanut butter baited A24s and those A24s that are angled in trap stands should improve house mouse trapping

success when these traps are used operationally. We expect that results from our study will help guide property owners, community members, and practitioners that experience problems with house mice. However, field studies are a recommended next step to determine A24 effectiveness against wild mice populations, with the goal of developing the most efficient use practices and methodologies (e.g., trap spacing and area, bait/lure type, vertical/angled trap configuration, mouse density effects, nontarget species hazards). A24 traps could be a promising tool to help solve mouse problems while providing an alternative to toxic rodenticide uses and possibly the high labor costs associated with single-catch traps.

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Authors' contribution

ABS conceptualized and designed the study, acquired the funding, obtained ethics approval; all authors collected the data; ABS analyzed the data and wrote the original draft; all authors reviewed and edited the manuscript.

Ethics and permits

All aspects of this project were approved under the USDA National Wildlife Research Center's Institutional Animal Care and Use Committee (IACUC) study protocol QA-2995. Mention of a company or commercial product does not mean endorsement by the U.S. government.

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