Special Report

# Evaluation of two methods for mass euthanasia of poultry in disease outbreaks

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Outbreaks of **exotic Newcastle disease (END)** and **low pathogenic avian influenza (LPAI)** in the United States forced the euthanasia of millions of birds during 2002 and 2003. Both diseases are caused by viral pathogens that can infect most species of birds. The viruses are highly contagious and infectious and may cause no clinical signs or clinical signs of mild (clinical signs associated with the respiratory system for LPAI and those associated with the respiratory, nervous, and digestive systems for END) to severe disease with high mortality rate. The severity of disease depends on multiple variables, including the strain of the virus, the species affected, host factors, and environmental stressors. The viruses spread to susceptible avian hosts primarily through direct contact with infected birds but also through indirect contact with contaminated equipment and materials. Feces and secretions from the eyes, nose, and mouth of infected birds contain the viruses. The LPAI outbreak in March 2002 was first detected in commercial poultry flocks in the Shenandoah Valley region of Virginia.[1] In October 2002, END was initially detected in small, backyard poultry flocks in California.[2]

In both outbreaks, it was determined that the most appropriate course of action to gain control and prevent widespread dissemination was to immediately quarantine and euthanatize all flocks with positive results for either disease. A positive flock was defined as having clinical evidence of infection and positive results for at least 1 screening test. Also, if there was a clear epidemiologic link between a positive flock and a negative flock, the negative flock was depopulated. During the END outbreak, it was also necessary to depopulate negative birds in neighborhoods where epidemiologic data indicated that spread of the virus was imminent. The potential for severe economic effects caused the poultry industry along with the federal and state governments to launch a swift and thorough response to the outbreaks. The goal during these outbreaks was to euthanatize all birds on quarantined farms within 24 hours of a positive test result, when logistically possible. If epidemiologic data indicated the need for euthanasia, the goal was also to complete the euthanasia within 24 hours of the epidemiologic determination. Also, to minimize potential spread of the viruses, all euthanasias were performed on-site and carcasses were carefully contained for transport to disposal sites.

Determining the best way to euthanatize birds in large flocks was problematic for many reasons. The number of poultry on any premises can vary, depending on the type of operation and the number of poultry houses affected. Difficulties included adjustments for variation in the size of birds; variation in the size and type of housing facility; lack of a sufficient number of employees experienced in mass euthanasia; emotional and physical worker fatigue; locating temporary employees and providing them with appropriate training; communicating with employees whose native language was not English; maintaining employee morale in difficult circumstances; employee safety; biosecurity issues; the imposed time limitations; and lack of a proven method of humane and efficient mass euthanasia, given the constraints of the outbreak control policies.

The purpose of the report described here is to provide information concerning 2 successful methods of mass euthanasia of poultry. Mass euthanasia, in this context, refers to the euthanasia of poultry in houses containing thousands or tens of thousands of birds. A successful mass euthanasia was defined as one that involved humane handling of poultry, delivery of the euthanasia agent in quantities sufficient to induce anesthesia rapidly, and a 100% mortality rate promptly after anesthetic induction.

## Mass Euthanasia Method Planning

**Choosing the euthanasia agent**—The universally accepted, authoritative document on euthanasia is the *2000 Report of the AVMA Panel on Euthanasia.*[3] Acceptable methods (eg, those that consistently result in a humane death when used as a sole means of euthanasia) for euthanasia of birds include barbiturates, inhalant anesthetics, **carbon dioxide ($CO_2$)**, carbon monoxide, and, for free-ranging birds, gun shot.[3]

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Cervical dislocation is listed as a "conditionally acceptable method" (eg, "…those that by the nature of the technique or because of greater potential for operator error or safety hazards might not consistently produce humane death or are methods not well documented in scientific literature"[3]) and was therefore only used on small birds (approx 0.91 kg [2 lb]) as a secondary method during euthanasia of large flocks.

The *2000 Report of the AVMA Panel on Euthanasia*[3] acknowledges that in disease eradication efforts, euthanasia options may be limited. In the LPAI and END outbreaks, options were limited by the large number of poultry involved, the various types of housing used for poultry (depending on the type of poultry flock), consideration for employee safety, and biosecurity concerns, which had prompted the policy for on-site euthanasia within 24 hours of a positive test result. The *2000 Report of the AVMA Panel on Euthanasia*[3] was used as a guideline in accordance with the professional judgment of state and federal departments of agriculture and industry stakeholders to determine the methods of euthanasia. The basic principle of euthanasia (eg, that it should be painless and minimize stress and discomfort) was, and should always remain, the primary consideration. Development of euthanasia methods that addressed this principle, but could also work within the reality of the environment and situations in which euthanasia had to be performed, was imperative. The methods described herein were used to euthanatize several thousands or tens of thousands of birds in individual poultry houses.

The agent chosen for mass euthanasia was compressed $CO_2$ gas. Injectable barbiturates would require handling of birds individually and also had the disadvantages of potential misuse, being controlled substances, being expensive, and requiring a certain level of skill to humanely inject each bird. Inhalant anesthetics and carbon monoxide involve a high safety risk to employees, and because birds were confined, gunshot was inappropriate.

The rapid analgesic and anesthetic properties of $CO_2$ are well known. Carbon dioxide concentrations as low as 7.5% increase the pain threshold.[3] The mode of analgesic and anesthetic action of $CO_2$ is induction of hypercapnic hypoxia.[4] Hypoxia induction blocks those parts of the brain (eg, cerebral cortex and subcortical structures) that are responsible for pain processing. Murine cholinergic markers are not distorted, and corticosterone concentrations are not changed by exposure to $CO_2$, supporting the idea that distress in animals is limited when $CO_2$ is used for euthanasia.[5] Other advantages of the use of $CO_2$ are that it is relatively inexpensive, compared with injectable euthanasia agents or even argon and nitrogen gases, and because it is nonflammable and nonexplosive, the safety hazard for personnel is low. Risk to personnel was not considered minimal because of the potential of exposure to high $CO_2$ concentrations in an enclosed environment. Results of several studies[6,7] indicate that inhalation of high $CO_2$ concentrations may cause signs of distress in certain animals. Additionally, low $CO_2$ concentrations reportedly cause nasal mucosa and pulmonary lesions,[6,7] although a high level of humidity, as was usually observed during euthanasia of poultry, decreases the amount of irritation of the airway mucous membranes.[8]

**$CO_2$ concentration and use—**The molecular weight of $CO_2$ is 44.01, which is 50% heavier than air. Its weight allows it to quickly displace air near the ground, at a level close to the breathing height of birds being housed on litter. Carbon dioxide concentrations of 50% to 55% for euthanasia of adult chickens have been recommended.[9] This percentage was determined by studying various $CO_2$ concentrations and the time required for loss of brain function, as measured by **electroencephalograms (EEGs)** and somatosensory evoked potentials. At concentrations of approximately 45% $CO_2$ in air, introduced during a period of 18 seconds, EEGs are suppressed in 21 seconds and quiescent at a mean of 30 seconds with death occurring in 2 to 5 minutes in adult chickens.[10,11] Turkeys require a longer duration of exposure for the loss of brain responsiveness, indicating that turkeys may be more tolerant of hypercapnic hypoxia than chickens[6] and therefore may require longer periods of exposure to $CO_2$. At 65% $CO_2$, turkeys lost somatosensory evoked potentials in 14 to 32 seconds and brain function (as indicated by isoelectric EEGs) in 57 to 95 seconds.[7]

Calculations to determine the amount of $CO_2$ required to reach appropriate concentrations were complicated by the anticipated leakage of enclosures built quickly on-site. Also, the volume of space taken up by poultry in the enclosure and the air trapped in plumage and between birds were expected to cause dilution effects, resulting in further variability when estimating the amounts of $CO_2$ required. It was considered essential that the $CO_2$ concentration reached reliably caused death in all birds; therefore, calculations to determine the amount of $CO_2$ required were based on obtaining an estimated final concentration of 70% $CO_2$. It was thought that this would allow some room for potential complications, such as an error with calculations or a malfunctioning gas tank, and would permit a target concentration of 50% to 60% $CO_2$ to be reached. To calculate $CO_2$ requirements, the volume of the empty enclosure to be used for euthanasia was first determined (eg, length $\times$ width $\times$ height). This number (in cubic feet) was multiplied by 70% $CO_2$ to assure the targeted 50% to 60% $CO_2$ concentration would be obtained. One pound of $CO_2$ generates approximately 8.75 cubic feet of gas at 1 atm of pressure and at a temperature of 21.1°C (70°F). Therefore, the formula for quick calculation of the amount of $CO_2$ required was as follows: 70% $\times$ (1 lb of $CO_2$/8.75 cubic feet) = 0.08 lb of $CO_2$/cubic feet. Although this calculation was an estimate, it was effective. The complications involved and the absence of reported $CO_2$ measurements from previous mass euthanasia procedures made exact calculations difficult. Erring on the side of excess $CO_2$ was considered to be more humane than not having enough $CO_2$.

It has also been suggested that using certain gas mixtures (eg, argon and nitrogen) may provide a more humane death for poultry (ie, decreased signs of discomfort and decreased physical reaction from exposure to the gas). However, the enclosures needed to provide the proper environment to use these gases would need to be almost airtight. Although this approach was discussed as an option, the absolute containment needed to maintain the necessary exacting gas proportions was not feasible in this field situation. Maintaining the constant specific 2% to 5% **oxygen ($O_2$)** concentration[10] that was needed in the mixtures was not possible. Even if an airtight enclosure was constructed, developing a way to adequately mix gases within the enclosure would have been difficult and limited time was available for conceiving and developing a workable process. Fluctuations in the $O_2$ concentrations while using a gas mixture could result in the recovery of consciousness in birds.[12] Also, the ability to immediately obtain the volume of gases required for the mixtures was not certain. Given the confounding factors of these outbreaks, it was determined that $CO_2$ alone was the best choice for the euthanasia agent.

## Other Models for Euthanasia

**Controlled atmosphere stunning (CAS)** and **modified atmosphere killing (MAK)** are 2 types of systems that use gases in specially built chambers to anesthetize or euthanatize poultry.[10,12-14] These types of systems are being used on farms in some countries to dispose of hens that are no longer productive (ie, spent) and in processing plants to initially stun poultry. As more information becomes available, these types of systems may prove to be a humane option for euthanasia during disease outbreaks. A few units[a,b] are commercially available, but none of the mobile units were large enough to be used in the outbreaks of this report. The MAK system holds 200 to 250 hens and can be rolled down the aisles between cages in a poultry house.[15] This system may be useful for euthanasia of poultry in the future, although method 2 in the report described here uses a similar concept and 350 to 400 birds could be euthanatized at 1 time. The CAS system uses a conveyor belt approach in which poultry are moved through adjacent chambers consisting of different mixtures and concentrations of gases for stunning. Controlled atmosphere stunning and MAK are promising technologies for humane processing of poultry and disposing of birds that are no longer efficient producers and may be effective in managing disease outbreaks in poultry.

## Ground Panel Enclosure (Method 1)

Although systems or methods that are humane, reliable, easy to disinfect and transport, rapid to set up and use, and safe for employees must continue to be developed, 2 successful methods of mass euthanasia are considered here. In the first method, a flock with positive results for LPAI was used to assess a procedure for mass euthanasia developed by personnel from Animal and Plant Health Inspection Service Animal Care and Veterinary Services and the Shenandoah Valley poultry industry. The test flock consisted of 4,200 ten-week-old turkeys weighing approximately 3.6 kg (8 lb). All turkeys were housed in 1 building.

A rectangular enclosure (56 × 24 × 4 feet) made of plywood sheets braced along its exterior by 2 × 6-inch boards was constructed at 1 end of the turkey house. One 24-foot side of the enclosure was left open so the turkeys could be walked into the enclosed space. Rope was positioned in a zigzag pattern across the 24-foot sides of the enclosure to support a tarp ceiling. A ceiling was necessary to contain the $CO_2$, and the rope prevented excessive sagging of the tarp. Duct tape was used to seal the outside junctions between the plywood sheets. Poultry house litter was pushed up against the bottom of the boards to seal any gaps. The 60-foot side of a 36 × 60-foot tarp was secured on one of the 56-foot enclosure sides by bungee cords. Enough rope was then tied to the 2 nonsecured 36-foot corners of the tarp, allowing the tarp to be manually pulled over to the opposite side of the enclosure. A tarp ceiling could then be created after the turkeys had entered the enclosure. The tarp was gathered in a fan-fold fashion and positioned on the secured side. A large (387-lb) cylinder of $CO_2$ was manually placed a quarter of the way into the enclosure and set next to one of the plywood panels with the outlet nozzle of the tank facing into the enclosure. Construction of the enclosure was completed in 2 hours by approximately 7 people.

After the enclosure was completed, all 4,200 turkeys were moved into the enclosure and the entry-

Table 1—Summary of data obtained before and during mass euthanasia of a flock of turkeys with low pathogenic avian influenza (prototype) or 3 flocks with exotic Newcastle disease by use of carbon dioxide ($CO_2$) in a ground panel enclosure (method 1).

| Variables | Flock 1 | Flock 2 | Flock 3 | Prototype |
|---|---|---|---|---|
| Maximum $CO_2$ concentration (%) | 57.0 | 47.5 | 57.5 | Not measured (62 theoretical) |
| Time to reach maximum $CO_2$ concentration (min:s) | 6:0 | 5:0 | 6:0 | Not measured |
| Time to reach $CO_2$ concentration of 50% (min:s) | Approximately 4:45 | NA | Approximately 4:30 | Not measured |
| Time to no sound or movement (min:s) | 6:0 | 6:0 | 7:0 | 10:0 |
| Enclosure size (feet) | 32 × 40 × 4 | 32 × 40 × 4 | 32 × 40 × 4 | 24 × 56 × 4 |
| No. of turkeys | 5,500 | 5,500 | 5,500 | 4,200 |
| Approximate weight of turkeys (lbs) | 12 | 12 | 12 | 8 |
| Source of $CO_2$ | Truck | Truck | Truck | 387-lb cylinder |

NA = Not applicable.

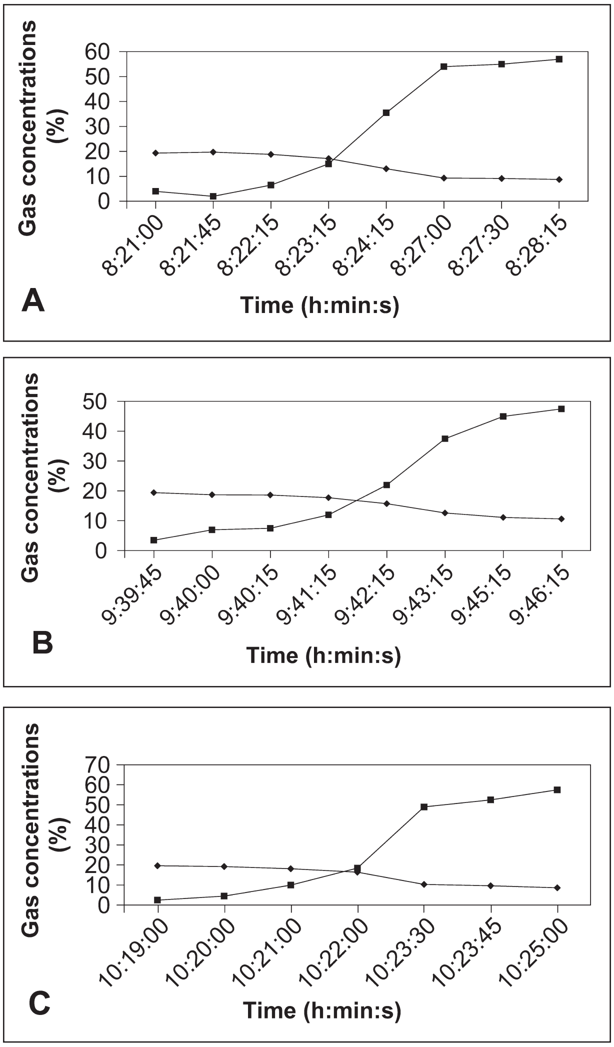


Figure 1—Concentrations (%) of carbon dioxide ($CO_2$; squares) calculated from measurements of oxygen concentrations (diamonds) obtained during mass euthanasia of 3 flocks of turkeys (A, B, and C; n = 5,500 turkeys in each flock) with exotic Newcastle disease by use of $CO_2$ in a ground panel enclosure (method 1).

way was closed with additional plywood sheets. The tarp was pulled over the top of the enclosure and was large enough to permit a 2-foot minimum overhang on all sides. Once in place, the tarp was secured with bungee cords or remained in place as a result of its own weight. Placement of the $CO_2$ cylinder easily permitted manual manipulation by the valve handle being grasped from outside of the enclosure with the tarp draped over it. The valves were slightly opened on the $CO_2$ cylinder, permitting the turkeys to adjust to the sound of the escaping gas. After approximately 1 minute, the valves were fully opened. Although $CO_2$ is odorless, it was easy to determine if there were gross leaks by visually checking the tarp. Gross leaks around the tarp edges resulted in a waving motion of the tarp; when this was detected, the leak was rapidly secured with duct tape.

A few modifications of method 1 were used for successful mass euthanasia of poultry. One modification was used during euthanasia of a flock of broiler breeder chickens housed on raised, slatted floors. The poultry house had an open alleyway that was approximately 6 feet wide, extending down the center of the building. Nesting boxes lined both sides of the alleyway. Plywood panels were placed down the length of the alleyway on both sides and rested against the nesting boxes. The panels were slightly overlapped and quickly and easily secured with nails. Six 50-lb $CO_2$ cylinders were moved into the alleyway and secured to the panels, and the chickens were herded into the area. Several tarps were pulled over the top of the panels to create a ceiling, and the gas was released from the cylinders. Although the enclosure was not a formally constructed arrangement, it was an easy modification of method 1 that was successfully used for mass euthanasia of poultry.

Table 2—Concentrations of $CO_2$ calculated from measurements of oxygen ($O_2$) concentrations obtained at 2 points during release of $CO_2$ into an empty metal chamber enclosure (method 2) for mass euthanasia of poultry.

| Minutes after $CO_2$ release | Concentration of $CO_2$ at low point (%) | Concentration of $CO_2$ at high point (%) |
|---|---|---|
| 1 | 52.5 | 51 |
| 2 | 62.5 | 60.5 |
| 3 | 65 | 65 |
| 4 | 67 | 67.5 |
| 5 | 69 | 70.5 |
| 6 | 70.5 | 71 |
| 7 | 71.5 | 71.5 |
| 8 | 72 | 71.5 |
| 9 | 72.5 | 72.5 |
| 10 | 73 | 73 |
| 18 | 75 | 75 |

Oxygen concentrations were measured by use of an $O_2$ sensor,[c] and the concentration of $CO_2$ was calculated (a 1% decrease in $O_2$ concentration corresponds to a 5% increase in $CO_2$ concentration).

Another modification of method 1 involved lowering the tarp during euthanasia. After wing flapping (visualized by the waving of the tarp as well as being heard) and vocalizations could no longer be heard coming from the enclosure, the tarp was lowered to a height that would have been at the level of the head of a standing bird. It was believed that this procedure would decrease the volume of space to be filled by $CO_2$ and thus further increase the concentration of $CO_2$ at the birds' breathing level.

## Metal Chamber Enclosure (Method 2)

Poultry housed in cages require a different method for euthanasia because there is no room to construct an enclosure of any substantial size. A metal euthanasia chamber that could be placed over a flat of poultry was constructed. A flat holds approximately 350 to 400 birds and is used to transport poultry to processing plants. Experienced personnel (catchers) are employed to move birds from cages into these flats.

A prototype metal chamber measuring 10 × 6 × 5.3 feet was constructed. The chamber was built so that a gas cylinder could be connected for $CO_2$ delivery into the interior. A 0.5-inch-diameter hole was drilled into the top of the prototype chamber to permit venting of chamber air while the enclosure was filling with $CO_2$. Two channels were welded to the top of the chamber to allow it to be moved with a forklift, which was used to place the chamber over the flat and remove it when finished. Two small holes were drilled into 1 side, 1 several inches from the top of the chamber and 1 at the height of the lowest flat. These holes permitted monitoring of gas concentrations. The holes were purposely located on the side farthest away from the $CO_2$ delivery site. Oxygen concentrations were measured by use of an $O_2$ sensor,[c] and the concentration of $CO_2$ was calculated (a 1% decrease in $O_2$ concentration corresponds to a 5% increase in $CO_2$ concentration). Although it would have been preferable to measure $CO_2$ concentrations directly, an $O_2$ sensor was used because a $CO_2$ sensor that could withstand repeated exposures to high concentrations of $CO_2$ without malfunctioning was not available.

## Results

In the method 1 trial run (prototype), a 387-lb cylinder of compressed $CO_2$ was used. Each pound of $CO_2$ provides approximately 8.75 cubic feet of $CO_2$ gas at 1 atm of pressure and at a temperature of 70°F. Therefore, this cylinder should have provided approximately 3,386 cubic feet of 100% $CO_2$. The volume of the enclosure was calculated to be 5,376 cubic feet. The percentage of $CO_2$ in the enclosure, without taking into account the volume of space occupied by the 4,200 birds and assuming complete emptying of the cylinder, would have been approximately 62% (3,386 cubic feet of $CO_2$/5,376 cubic feet of air; **Table 1**). For the trial run, the cylinder was allowed to completely empty, taking approximately 20 minutes. Personnel could hear wing flapping as well as see a rippling movement of the tarp and hear an increase in vocalizations within approximately 1 minute of opening the $CO_2$ cylinder. Five minutes after opening the cylinder, wing flapping had almost completely stopped, and 10 minutes after opening the cylinder, no wing flapping could be heard and the tarp was not moving. After the cylinder had emptied, the tarp was left in place for an additional 15 minutes to ensure euthanasia. During that time, no sounds were heard from the enclosure. When the tarp was removed, a 100% mortality rate had been achieved.

Although no gas concentrations were measured during the trial run for method 1, $O_2$ concentrations were measured during use of a slightly modified method 1 procedure for mass euthanasia of 3 flocks of turkeys with END (**Figure 1**). Modifications to method 1 included use of a truck for $CO_2$ gas delivery and an enclosure size of 40 × 32 × 4 feet (5,120 cubic feet). In each flock, five thousand five hundred 12-lb turkeys were euthanatized. An $O_2$ sensor[c] was used to measure the $O_2$ concentrations at various sites inside the enclosure, close to the periphery. Measurements obtained from the center of the enclosures would have been useful, but positioning a sensor in that location was not feasible.

The calculated concentrations of $CO_2$ indicated that it was possible to reach a target concentration of 50% to 60% by use of method 1 in approximately 6 minutes in a volume of 5,120 cubic feet. The time from $CO_2$ exposure to a lack of movement and sound from the enclosure (indicating deep anesthesia or death) was ≤ 7 minutes during euthanasia of the 3 flocks with END.

In method 2, a trial run was performed to determine the distribution of $CO_2$ in the metal chamber enclosure when it was placed over an empty flat. An $O_2$ sensor[c] was used to measure the $O_2$ concentrations inside the chamber while $CO_2$ was being released from an attached 50-lb cylinder. The area around the sensors was sealed with duct tape. Oxygen concentrations were obtained every minute for approximately 20 minutes (**Table 2**). Measurement of the concentration of $O_2$ in the room air was 20.09%, and the $CO_2$ concentration was negligible, as would be expected. These measurements were obtained to provide evidence that the sensor was working properly. Concentrations of $CO_2$ were calculated in the same manner as for method 1. The cylinder emptied in 7 minutes. The $CO_2$ concentrations calculated from measurements of the $O_2$ concentrations obtained from the sensors positioned near the top and bottom of the enclosure were almost identical. A concentration of approximately 52% $CO_2$ was reached in the chamber 1 minute after $CO_2$ was released from the cylinder, and concentrations > 52% were maintained for a period of 18 minutes.

Method 2 was used during mass euthanasia of 3 flocks of chickens with END, and the concentration of $O_2$ was measured during each procedure (**Table 3; Figure 2**). The time required to reach the target concentration of 50% $CO_2$ was ≤ 1 minute. The time from $CO_2$ exposure to a lack of movement or sound was < 2 minutes.

Table 3—Summary of data obtained before and during mass euthanasia of 3 flocks of chickens with exotic Newcastle disease by use of $CO_2$ in a metal chamber enclosure (method 2) and during release of $CO_2$ into an empty (prototype) metal chamber enclosure.

| Variables | Flock 1 | Flock 2 | Flock 3 | Prototype |
|---|---|---|---|---|
| Maximum $CO_2$ concentration (%) | 80.0 | 71.7 | 71.5 | 75 |
| Time to reach maximum $CO_2$ concentration (min:s) | 4:30 | 1:15 | 4:00 | 18:0 |
| Time to reach $CO_2$ concentration of 50% (min:s) | 1:0 | 0:45 | 1:0 | 1:0 |
| Time to no sound or movement (min:s) | 1:45 | 1:40 | 1:0 | NA |
| Enclosure size (feet) | 10 × 6 × 5.3 | 10 × 6 × 5.3 | 10 × 6 × 5.3 | 10 × 6 × 5.3 |
| No. of chickens | 375 | 375 | 375 | None |
| Size of $CO_2$ cylinder (lb) | 50 | 50 | 50 | 50 |

NA = Not applicable.

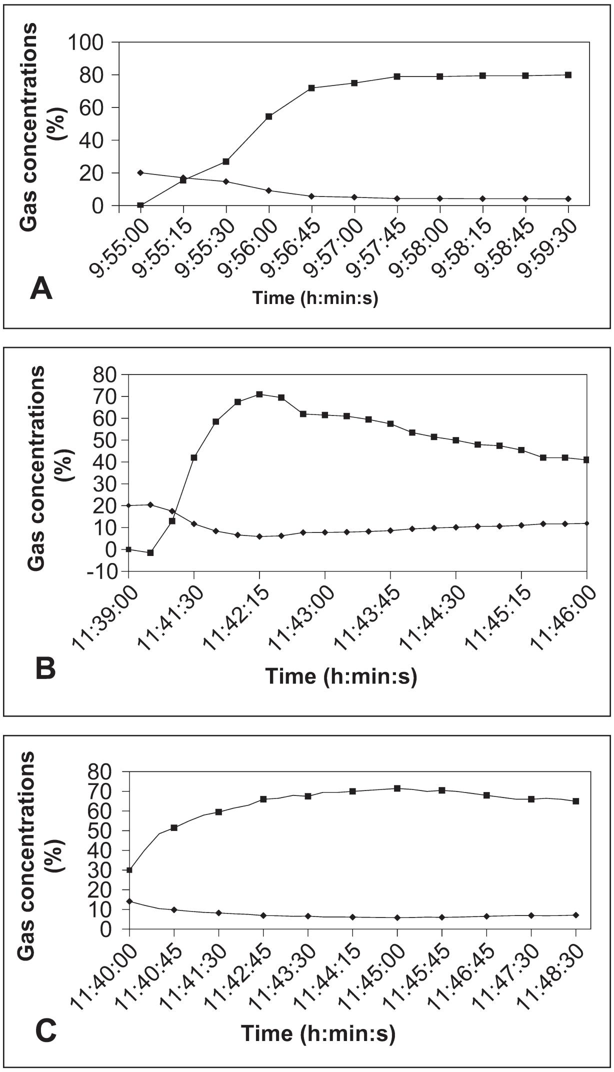


Figure 2—Concentrations (%) of $CO_2$ (squares) calculated from measurements of oxygen concentrations (diamonds) obtained during mass euthanasia of 3 flocks of chickens (A, B, C; n = 375 chickens in each flock) with exotic Newcastle disease by use of $CO_2$ in a metal chamber enclosure.

## Discussion

By use of either method of euthanasia in the report described here, it was possible to obtain the targeted concentrations of $CO_2$ in a timely manner, as required for humane mass euthanasia. The maximum concentration of $CO_2$ reached during use of method 1 was within (57.0% and 57.5%) or close to (47.5%) a target concentration of 50% to 60% $CO_2$. Although approximately 4 and a half minutes was required to reach the targeted concentrations of $CO_2$, the time to no sound or movement from the enclosures occurred shortly (approx 120 seconds) thereafter, indicating anesthetic concentrations of $CO_2$ were quickly reached. The gradual increase in $CO_2$ permits time for the birds to adjust to the smell of $CO_2$ and the loud sound of $CO_2$ being released from the cylinders or delivery truck hoses. It also permits induction of analgesia and unconsciousness prior to exposure to high, potentially caustic $CO_2$ concentrations.[16] In the trial run for method 1, when the tarp was removed, it was noticed that the birds had remained in a fairly uniform, single layer, indicating that birds did not have signs of panic and did not pile up on one another, which may be associated with an attempt to escape from a noxious smell or fearful sound.

The concentrations of $CO_2$ (80.0%, 71.7%, and 71.5%) obtained during use of method 2 were much higher than with method 1. This would be expected because the metal chamber did not leak and the square footage of the chamber was smaller, compared with enclosures used in method 1. Results also indicated that the $CO_2$ concentrations necessary for inducing analgesia, unconsciousness, and death were rapidly reached in method 2.

Induction of hypoxia has been found to cause motor activity in birds, including wing flapping, vocalization, and seizures.[17] Vocalization and wing flapping were heard in both methods and also detected in method 1 (as evidenced by the waving of the tarp in concurrence with the sound of the wing flapping); however, use of opaque tarps and solid-sided chambers interfered with further observations in these methods. Although aesthetically unpleasant, this motor activity follows loss of consciousness, is a reflex activity, and therefore is not perceived by the animal.[11,18] In the authors' experience with these euthanasia methods, intense wing flapping began within a few minutes of gas delivery. Assuming $CO_2$ concentrations were reached as calculated and indicated by the limited data, the timing of motor activity indicated that birds would have been unconscious during the wing flapping and the activity was unlikely to have been caused by distress. Time to unconsciousness and $CO_2$ levels must be verified during future mass euthanasia procedures.

In method 1, after motor activity had stopped, the tarps were left in place for times that may have been longer than were necessary, typically 15 minutes. However, the respiratory physiology of poultry makes it possible for them to recover remarkably quickly after gas stunning.[19] In 45% $CO_2$ for 2 minutes, broiler chickens responded to comb pinch 26 seconds after the gas was removed.[9] Permitting poultry to remain in the concentrated $CO_2$ environment for an additional 15 minutes so that birds did not recover consciousness was important. Further research is required to determine whether this additional 15-minute period after gas delivery can be decreased.

When the enclosure is formed by use of panels as in method 1, the size of the enclosure can be manipulated to adapt to the size of the poultry house and number and size of birds by adding or removing panels. Although the time required for construction of the enclosure may seem long (2 hours), this time may be decreased with practice and a better system of connecting the panels. The prototype enclosure used in the trial run for method 1 was made with wood; however, metal panels were developed for use after successful use of the prototype. The metal panels were equipped with hinges on each end that interlocked with the hinges from adjacent panels. Metal rods were passed through the interlocked hinges to connect the adjacent panels. Method 1 can be used in almost any situation in which poultry are housed on litter.

Method 2, or a modification of this method, is best used in facilities in which poultry are housed in cages, such as with commercial egg-laying flocks. Although the metal enclosures worked well for euthanasia and could be sanitized and reused, a forklift was required to move the enclosures. Specially constructed chambers are quick and easy but are not required for euthanasia of poultry in flats. For euthanasia of many flocks with END, poultry were placed into flats, plastic was wrapped around the flat, leaks were sealed with duct tape, and $CO_2$ was introduced through the plastic. When this modification was used, it was imperative that personnel worked quickly and efficiently so that birds were not stressed by the heat and humidity generated within the sealed enclosure. Death caused by suffocation rather than hypercapnic hypoxia is not appropriate.

Humane euthanasia of poultry was an important consideration during evaluation of welfare concerns. Death by suffocation or heat stress must not be considered a successful outcome, even though death was the end result. Attention to detail, appropriate handling of poultry, and following the principles reported here, regardless of enclosure configuration or type, are important for ensuring successful euthanasia.

One of the advantages in method 1 is that poultry do not undergo the stress of being handled. Poultry tend to clear a path when humans walk toward them; thus, it was not difficult to herd a flock into an enclosure without causing excessive stress to birds. When movement into the enclosure was performed appropriately, the welfare of the birds was not compromised. Although on-site euthanasia was chosen as a biosecurity measure, this also removed the additional stress of transportation, which is particularly difficult for weak or sick birds.

When birds must be handled during euthanasia, welfare issues become a concern. Careless handling can cause distress and discomfort, especially for laying hens. Laying hens must be handled carefully because their bones are fragile.[20] Personnel must be carefully trained in appropriate handling techniques. During on-site euthanasia, temperatures can be uncomfortable for humans wearing layers of personal protective equipment, concentrations of ammonia in the environment may be high, the environment may be dusty, the work is physically tiring, and the situation is not conducive to maintaining high morale. These factors may cause personnel to handle birds roughly because of frustration or fatigue. Supervision is always important so that careless or inappropriate actions can be quickly corrected and kept from recurring.

Although measurements of heat or humidity within the enclosures were not obtained during the mass euthanasia procedures, gathering a large number of birds tightly into an enclosure and covering it with an impermeable tarp generates a tremendous amount of heat and humidity quickly. A few employees placed their face or arms beneath the tarp used in method 1 to evaluate welfare of birds during the euthanasia process. The temperature detected by these individuals was described as being uncomfortably warm, and there was concern that the heat generated would result in death from suffocation. A strong ammonia odor was also detected. To avoid causing undue discomfort to birds, all procedures must be organized before birds are moved into an enclosure. Detailed planning was necessary to make the process move quickly and ensure animal welfare.

## Lessons Learned and Experience Gained

Several lessons were learned during the process of determining the best way to humanely euthanatize large numbers of birds of various sizes housed in different settings. Use of high-quality duct tape to seal gaps and leaks was essential. Construction of the enclosure used in method 1 was simplified by use of lightweight material for the sides and rods as connectors. Enclosure materials that can be easily disinfected and moved are important considerations. During the trial stage of method 1, plywood was used because it was easy to obtain and inexpensive. However, the porous surface of wood made disinfection impossible, and the wood must be burned on-site to prevent it from acting as a fomite. Other lightweight but sturdy materials that can be completely disinfected could be considered for use as an enclosure. Variability in the height of the enclosure so that it could be adjusted to the height of birds would have been useful. An enclosure with low sides would require less gas, permitting quick accumulation of $CO_2$ and, therefore, quick euthanasia of birds.

The nature of managing an emergency situation precludes the planning of a true scientific study. The $O_2$ sensor used during the procedures reported here was not made to function in an environment of high $CO_2$ concentrations; however, $CO_2$ sensors that would work reliably in such an environment were also not available. Ideally, when used in that type of environment, the $O_2$ sensor should be replaced after 1 or 2 readings according to manufacturer's instructions. Changing the sensor frequently during the euthanasia procedures was not possible. To the authors' knowledge, the procedures reported here were the first in which the concentration of $O_2$ has been obtained, and when the 2 methods were performed in the manner described here, mass humane euthanasia was accomplished.

Finding a tarp large enough to completely cover some of the large enclosures used in method 1 was difficult. In those situations, tarps were combined with high-quality duct tape. The combined tarps withstood the rigors of the process without developing leaks, as long as high-quality duct tape was used.

Much was learned about the use of large amounts of $CO_2$. Prior familiarity with gas cylinder operation and the physical properties of $CO_2$ permits a procedure to be performed smoothly. Hoses should not be used to deliver $CO_2$ into an enclosure, or if they must be used, only use large-diameter hoses. Hoses freeze within minutes, and the euthanasia process will be delayed, causing birds to have signs of stress and discomfort. The most successful euthanasia procedures were those that released gas directly from cylinders without hoses to impede delivery. Increased efficiency in delivery of $CO_2$ was not detected when $CO_2$ was delivered by truck; this is believed to have been because hoses were used for delivery of $CO_2$ into the enclosure. One modification used during mass euthanasia of a flock with END involved use of hoses that resisted freezing. Even without hoses, when cylinder valves are fully opened, the cylinder itself may freeze and cause delays; therefore, knowledge of cylinder operation including appropriate flow rates to prevent freezing is necessary.

In the report described here, $CO_2$ cylinders in 50- and 387-lb sizes were used for mass euthanasia. Advantages of the use of 50-lb cylinders included employee safety (most of the time, cylinders were moved manually), increased availability from suppliers (this size cylinder is commonly used in fountain drink dispensers), and better distribution in the enclosures because the gas was released from multiple sources and because the cylinders could be physically positioned to permit uniform gas delivery throughout the enclosure. Positioning small cylinders ≤ 10 feet apart appeared to give consistently uniform gas dispersal. The 50-lb cylinders would also completely empty in 5 to 7 minutes and were less likely to freeze than the large cylinders. Additionally, if 1 cylinder failed during the procedure, other cylinders in the enclosure would have been able to provide enough $CO_2$ to compensate for the loss.

When gas cylinders were positioned in the enclosure with low sidewalls, the tarp ceiling needed to be supported over the area of the outflow nozzle; otherwise, the released gas would immediately encounter a sagging tarp that may impede flow of the gas. Elevation of the tarp away from the valve outlet (eg, by use of a ladder) resulted in efficient release and uniform dispersal of $CO_2$.

Determining an optimal density of birds to be moved into an enclosure area is important and depends on the size of birds and the size of the enclosure. Too many birds in an enclosure results in piling of birds on one another and signs of anxiety, discomfort, and distress, and death for many may be attributable to asphyxiation. Herding too many birds into an enclosure permits some birds to escape by hopping onto one another and over the enclosure edge, which may lead to frustration of employees trying to catch loose birds, is time consuming, and can lead to inappropriate handling. Bird density is also important when considering how the tarp is to be pulled over the top of the enclosure. If the tarp is going to be walked across the enclosure by employees, rather than being pulled across by ropes, bird density must be such that employees can walk across the enclosure without stepping on birds.

Every enclosure should have a way to view the interior of the enclosure to permit monitoring of the euthanasia process. Use of clear plastic makes evaluation of the process easy; however, a viewing window should be incorporated into construction of the enclosure when opaque materials are used. The ability to visually monitor the euthanasia process permits accurate observations to be made, and problems can immediately be addressed.

Many areas remain to be investigated to improve the use of gas euthanasia for poultry, especially for large-scale emergency procedures. Although a few estimates of $CO_2$ concentration were determined at various points during the euthanasia procedures, a study of $CO_2$ concentrations measured from various areas of a large-volume euthanasia chamber needs to be thoroughly evaluated. Additionally, euthanasia by use of a gas mixture of $CO_2$ and $O_2$ may result in a quieter anesthetic induction, as compared to the use of $CO_2$ alone. An ideal mixture would cause immediate anesthesia without irritation and result in death in all exposed animals in a short duration.

There may be a maximum enclosure size after which dispersal of $CO_2$ is not adequate, given the size of the gas cylinders used. Use of multiple 50-lb cylinders was thought to provide a more even dispersal of the gas, compared with use of a 387-lb cylinder; however, this is another area that needs to be evaluated. Optimal flow rate for chamber euthanasia is reportedly one that displaces 20% of the chamber volume per minute.[21] Displacing 20% of the volume would permit an enclosure to be completely filled in 5 minutes. Measurements of flow volumes and $CO_2$ concentrations acquired in enclosures also require further evaluation.

Generation of heat and humidity and how quickly these parameters increase within the enclosure also need to be evaluated. During use of method 1, heat and humidity appeared to noticeably increase in the enclosure. Additionally, the concentration of ammonia appeared to be high after the tarp was pulled over an enclosure. These environmental aspects could cause distress to birds in the enclosure. Measurement of these factors will be important in ensuring animal welfare.

## Conclusions

Methods 1 and 2 describe procedures for mass euthanasia of poultry that were performed on-site, were humane and rapid, were inexpensive, and required a minimal number of employees. On-site euthanasia has the advantage of eliminating transport stress and causing few biosecurity risks. Observations that wing flapping began quickly after $CO_2$ release and ended rapidly indicated that birds became unconscious quickly and that death followed shortly thereafter. Oxygen concentrations measured indicated that effective $CO_2$ concentrations were rapidly obtained. A 100% mortality rate was achieved when procedures were closely followed.

Control of the LPAI outbreak in Virginia in 2002 affected 197 poultry production facilities and required euthanasia of 4.7 million birds,[22] most of which were euthanatized on-site. An additional 900,000 birds were sent to controlled slaughter. Mass euthanasia performed on-site was believed to be the best method for preventing further spread of the disease. The 2 methods for mass euthanasia in the report described here could be adapted to various situations. It is imperative that any refinement or change in methodology first be evaluated for animal welfare concerns.

The United States is the world's largest producer and exporter of poultry meat.[23] Americans also consume more poultry meat than either beef or pork.[23] Outbreaks of disease in poultry not only affects the industry and company employees, but also the consumer, as well as causing serious international trade ramifications, even if the disease is not on the Office International des Epizooties lists.[24] These outbreaks need to be addressed swiftly and thoroughly. Although a difficult task, it was determined that the best method for controlling the most recent LPAI and END outbreaks was to quarantine potentially affected flocks and to euthanatize birds on-site, ideally within 24 hours of a positive test result. When this method of eradication is chosen, planning and coordinating the process is challenging for many reasons. Each of these challenges is interesting; however, the purpose of the report described here was to provide information concerning successful methods of humane mass euthanasia of poultry.

Undoubtedly, disease outbreaks of a serious nature will continue to occur. When mass euthanasia is the method chosen for disease eradication, it is imperative that animal welfare is the utmost concern and the process is humane. Any method chosen must be able to withstand public and professional scrutiny. The USDA and poultry industry must take a proactive role in continuing to find and assure successful methods for mass euthanasia. Many options may be available for mass euthanasia during outbreaks of disease; however, additional information is required, and the information that is presently available needs to be widely disseminated.

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a. FPM modified atmosphere killing cart, FPM Inc, Fairbury, Neb.
b. Culling carts for the poultry industry, Seibring Manufacturing, George, Iowa.
c. OXOR II oxygen sensor, Bacharach Inc, New Kensington, Pa.

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