Special Report

Evaluation of carbon dioxide administration for on-site mass depopulation of swine in response to animal health emergencies

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Rapid methods for on-site swine depopulation are required in the event of an animal health emergency in North America. The term animal health emergency, as used in this context, includes a wide range of potential situations such as disease outbreaks, contamination with chemicals (eg, dioxin) or radionuclides (eg, cesium-137), and adverse animal welfare conditions created by transportation restrictions that severely limit feed deliveries and animal movement. As described by the AVMA, mass depopulation refers to methods by which large numbers of animals must be destroyed quickly and efficiently with as much consideration given to the welfare of the animals as practicable, but where the circumstances and tasks facing those performing depopulation are understood to be extenuating.[1] For example, in the event of a foot-and-mouth disease (FMD) outbreak in the United States, infected animals are to be humanely killed and disposed of ≤ 24 hours after diagnosis and all susceptible animals on adjacent farms within a specified radius are to be humanely killed and disposed of within 48 hours to limit viral replication and subsequent disease spread.[2]

These goals were not achieved by the former Ministry of Agriculture, Fisheries, and Food in the response to the 2001–2002 UK FMD outbreak. Prediction models used to evaluate FMD control methods estimated that, if all infected animals had been killed within 24 hours after the initial case report, the extent of the outbreak in the United Kingdom could have been reduced by 40%; further, if animals on contiguous farms had been killed within 48 hours as recommended, the outbreak could have been reduced by 66%.[3] The 2001 UK outbreak cost an estimated £8 billion (approx $13 billion) and reduced the British gross domestic product by approximately 0.2% in that year.[4] More recently, South Korea experienced a widespread outbreak of FMD during the last quarter of 2010 and first quarter of 2011, resulting in the mass depopulation of > 3.48 million animals, mostly hogs.[5] South Korean government officials admitted that there was a failure to handle the outbreaks at an early stage and reported that the disease cost the South Korean government 3 trillion won (approx $2.7 billion). Nearly 1.975 million people participated in the efforts to control the South Korean FMD outbreak, including 489,140 public servants and 338,862 soldiers; 8 public servants died, some reportedly due to exhaustion.[6] According to the USDA,[7] a likely cost of between $6 billion and $14 billion has been projected for a US FMD outbreak contained to California. In North America, a timely regional and national response to an FMD outbreak would be more difficult because of substantially greater numbers of animals and extensive interstate animal movement. For example, up to 600,000 pigs have been estimated to be in transit nationwide at any given time.[8] Both intra- and interstate animal movement would be quickly stopped with the declaration of an animal health emergency. Movement controls should help in limiting the spread of disease but would greatly increase the need for on-site culling owing to welfare reasons; space and feed issues would quickly become limiting factors as weanlings and nursery pigs (those between weaning and grower-finisher stages) could not be moved to depopulated nursery and finishing barns. In Manitoba alone, it has been estimated that an immediate closure of the United States–Canada border to animal transport would result in an immediate requirement to humanely kill about 10,000 pigs/d, 7 d/wk at the 2010 production level.[9]

Accepted euthanasia methods, such as captive bolt, gunshot, and lethal injection each require that individ-

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ual animals be handled and restrained, and that operators are properly trained in the correct application of the technique used. Given that large US swine operations commonly have ≥ 1,000 pigs in each building, many buildings on 1 farm site, and very few animal workers, handling individual animals would greatly slow the depopulation process and increase the potential for viral replication and spread in an FMD outbreak. Additionally, worker welfare and safety issues, including physical and emotional trauma, must be considered.[9] Clearly, faster and less labor-intensive methods would be required if the goals of timely control and disposal are to be met in the event of an FMD outbreak or other exigent situation. Depopulation and welfare culling of swine in commercial operations in the United States would likely take place on site following implementation of transport controls that limit animal movement.

Whole-house modified atmosphere killing methods, such as those described for poultry depopulation,[10–14] are not a viable option for use in swine production barns because of carcass removal impediments and technical issues (eg, freezing of gas cylinders and associated pressure-reducing regulators at high gas flow rates, timely delivery of effective gas concentrations, potential for freezing animals with cryogenic liquids or gas prior to loss of consciousness, and the need to generate extremely large volumes of ambient temperature gas from cryogenic liquids). On-site construction of dip-lift controlled atmosphere stunning systems such as those used for commercial stunning of swine, where animals are lowered into a high concentration of a stunning gas mixture prior to humane slaughter, is possible but deemed impractical to implement on site and not in keeping with the goal of developing a system that could be constructed quickly using locally sourced materials and labor.

One suggestion made from within the swine industry for on-farm depopulation is to introduce appropriate inhalant gases into enclosed dump-bed trucks, trailers, or temporary corrals constructed outside of production barns. Potential advantages of the use of inhalant gases over individually applied physical euthanasia methods for this purpose include the ability to rapidly move animals out of buildings via existing walkways, reduced individual animal handling and need for physical restraint during euthanasia, and the ability to simultaneously contain and kill many animals while providing a means for carcass containment and disposal transport. Unlike properly applied physical euthanasia methods, loss of consciousness with inhalant gas methods is not immediate. However, any distress or discomfort occurring prior to loss of consciousness must be weighed against the possible adverse effects that handling or restraining procedures required for the use of other methods may have on animal welfare.[12]

In this report, we describe development and evaluation of a method intended for on-site mass depopulation of pigs in an exigent situation. The method, which involves a gradual displacement application of CO_2 gas within enclosed chambers, was designed to rely on readily available and locally sourced materials and labor, to allow for pigs to be managed in groups rather than individually, and to avoid the repetitive actions necessary for large-scale implementation of traditional euthanasia methods, which can be hazardous and stressful for personnel.[9] Feasibility of the use of CO_2 for on-farm swine depopulation and the underlying principles governing the wash-in of gases into enclosed containers have been previously described.[15]

Rationale for Gradual Displacement Application of CO_2

It is well established that the use of CO_2 results in rapid depressant, analgesic, and anesthetic effects. Carbon dioxide is denser than air, which allows its use in open containers,[16] and the use of CO_2 as a preslaughter, controlled atmosphere stunning agent in pigs has been extensively studied.[17] Carbon dioxide gas is an AVMA- and American Association of Swine Veterinarians–approved agent for euthanasia of swine.[18,19] Carbon dioxide inhalation causes acute respiratory acidosis and subsequently produces a reversible anesthetic state by rapidly decreasing intracellular pH.[20,21] At a constant displacement rate of 20% of container volume/min, delivery of 100 vol % CO_2 produces a wash-in (inflow of CO_2 that purges container air) time constant of 5 minutes ([100% of container volume]/[20% of container volume/min]) for any size container and results in a mean CO_2 volume fraction of 63.5 vol % after 5 minutes of gas wash-in.[15,22]

The 2013 AVMA Guidelines for the Euthanasia of Animals recommend that CO_2 be introduced at a constant flow rate between 10% and 30% of the container volume/min for euthanasia in those species where aversion or distress can be minimized.[18] According to Raj and Gregory,[21,23] CO_2 concentrations < 30 vol % are not aversive to swine as determined on the basis of the pigs' willingness to enter this environment to access a food reward. Carbon dioxide gas produces a more rapid loss of consciousness, compared with an inert gas mixture of 70 vol % nitrogen-30 vol % CO_2, when administered by gradual displacement methods,[24] and gradual displacement administration of CO_2 induces unconsciousness before the gas reaches concentrations that have been reported to activate ocular and nasal mucosal nociceptors.[18] When CO_2 is administered to young pigs at a constant displacement rate of 10% or 20% of the container volume/min, unconsciousness occurs within 80 to 124 seconds at approximately 22 vol % CO_2 concentration; increases in plasma concentrations of cortisol, norepinephrine, and lactate following CO_2 administration are similar to those found following the AVMA-recommended physical euthanasia methods of captive bolt and electrocution,[24] implying equivalency of these methods. Unlike the inert gases nitrogen or argon, which must be kept within very tight concentration ranges to result in oxygen concentrations < 2 vol % for effective killing, CO_2 can render animals unconscious and result in death over a wide range of concentrations, even when the oxygen concentration is > 2 vol %.[12]

Our group has previously described other advantages of CO_2 for on-farm depopulation of swine, including its ready availability and relatively low cost, nonflammable and nonexplosive properties, a minimal hazard when used outdoors with properly designed equipment, and that (unlike other gases such as carbon monoxide) toxic effects due to accidental exposure of personnel to CO_2 can be readily reversed by prompt removal from the area.[15]

Preliminary Experiments (Dumpsters and Temporary Corrals)

We investigated the use of modified roll-off solid waste dumpsters for containment of adult pigs and smaller temporary corrals constructed for containment of weanling and nursery pigs during on-site depopulation. Roll-off dumpsters have flat, solid bottoms and sides, are locally available in substantial numbers from waste management companies, and can be moved from site to site with purpose-built trucks (**Figure 1**). In addition, roll-off dumpsters have a side-opening rear door that can be used to facilitate walking the pigs from a building into the container, and the structure can be enclosed by covering with clear polyethylene sheeting (**Figure 2**). Temporary corrals can be constructed on site with wire mesh fencing,[a] plywood flooring, and clear polyethylene sheeting to cover the floor, sides, and top (**Figure 3**).

Dumpster and corral mock-ups (ie, test chambers) were built at the North Carolina State University Lake Wheeler Swine Education Unit to refine and validate gas delivery methods prior to animal experiments. The dumpster mock-up test chamber was constructed on the concrete floor of a well-ventilated arena. The walls of this container were constructed of 1/4-inch thick plywood to duplicate the approximate inside dimensions of a standard (nominally) 30–cubic yard solid waste dumpster: 7 feet wide × 5 feet high × 21.5 feet long. The bottom edges of the plywood walls were placed on standard foam pipe insulation to create a seal where the walls met the concrete floor. The top of the container was covered with clear, 6-mil-thick polyethylene sheeting so that the outside walls were draped by sheeting for a distance of approximately 20 inches down from the top, and the bottom edges were secured to the walls

Figure 1—Photograph of a (nominally) 30–cubic yard roll-off solid waste dumpster and transport truck.[i] Internal dimensions are 7 feet wide × 5 feet high × 21.5 feet long. A 20–cubic yard dumpster has the same floor area with shorter side walls, which reduces the amount of CO_2 gas required when the dumpster is used in constructing a chamber for mass depopulation of pigs. The black tarpaulin is used for retention of materials during road transport and not intended or used for containment of gases.

Figure 2—Photographs of a (nominally) 30–cubic yard roll-off solid waste dumpster[i] modified to serve as an animal enclosure (chamber) in a study evaluating a gradual CO_2 displacement method for mass depopulation of pigs (A and B). A—An inclined wooden panel was used to span the gap between the floor of the chamber and the ground to serve as a loading ramp; the floor was subsequently covered with wood shavings (contained in covered barrels) to provide footing for the pigs. B—After loading, the door gaps of the chamber were sealed with spray insulating foam,[k] and clear, 6-mil-thick polyethylene sheeting was applied as a cover and secured with bungee cords. A wash-out vent was then cut at the centroid of the top cover (not shown). The cut comprised 3 sides of a square approximately 6 inches long on each side; the resulting flap was opened during CO_2 wash-in to allow gases (primarily air) to escape and then closed for the remainder of the 20-minute administration period. The CO_2 gas (20% of chamber volume/min for 5 minutes) was supplied through a 3-inch internal diameter, schedule 40 PVC pipe fabricated to hook over the side of the dumpster and extend down the inner sidewall to terminate 9.8 inches above the floor. Gas delivery was directed downward to the floor to maximize mixing with air and increase CO_2 concentration rapidly in the lower part of the chamber.[25]

Figure 3—Photographs depicting a temporary corral constructed on-site for testing of a gradual CO_2 displacement method for mass depopulation of pigs (A and B). The ground was covered with clear, 6-mil-thick polyethylene sheeting overlayed with plywood panels to prevent damage from hooves, and wood shavings were added to provide additional traction. Wire fence paneling was covered on the sides and top with the same sheeting, and the top and bottom sheet edges were rolled and held in place with sandbags to produce a tight seal. A wash-out vent was cut into the top cover and the delivery system for CO_2 gas was fabricated as described for the dumpster chamber.

with duct tape at intervals of approximately 3.3 feet. A wash-out vent was cut at the approximate centroid of the top cover; the cut comprised 3 sides of a square approximately 6 inches long on each side. This allowed the vent to have a flap which could be manually opened to allow gas, primarily air, to escape during CO_2 wash-in and then closed to retain the gas during the remaining administration period.

A 14–cubic yard temporary corral mock-up test chamber (8 × 3 × 16 ft) was constructed on-site next to a swine barn. The ground cover was clear, 6-mil polyethylene sheeting, with plywood panels placed over the sheeting to prevent damage from hooves; later testing indicated the ground cover sheet was not necessary if good sealing between the sides and the bare ground could be obtained (data not shown). Wire mesh fencing[a] was used to form the sides, and the top and sides were fully covered with the same 6-mil polyethylene sheeting. The edges of the cover sheet and bottom sheet were rolled together and secured with sandbags at the base of the structure to create a seal. A wash-out vent hole was cut in the cover as described for the larger test chamber.

Carbon dioxide administration methods were initially modeled and optimized by means of computational flow dynamic modeling[22] prior to animal experiments. For modeling of gas concentrations, flows, and mixing, 3-D models representing the 2 test chamber volumes and shapes were created with commercially available software programs.[b,c] These were basically thin-walled solid models with inside dimensions that matched the inside chamber dimensions; a top cover was included to represent the plastic film sealing the top of each chamber. Once the model of a given setup was created, the boundary conditions and initial conditions were specified by setting the pressures, flow rates, and compositions of gases at all openings. This included the CO_2 inlet pipe and the wash-out vent hole in the top cover. The CO_2 flow rate across the inlet pipe lid was specified to be equal to 20% of the total chamber volume/min, or 150 cubic feet/min for the 30–cubic yard chamber and 75 cubic feet/min for the 14–cubic yard chamber. Gas temperature can also be specified, which is important for predicting temperatures in the chambers when CO_2 is delivered at low temperatures; for these simulations, an inlet temperature of 0°C was used. The composition of the initial gas volume in the chamber was specified as air. A more detailed description of the computational flow modeling methods can be found elsewhere.[22]

Gas concentrations within each mock-up test chamber were confirmed with a CO_2 sensor[d] and an oxygen sensor.[e] The gas sampling system was controlled through a computer program.[f] Samples were drawn through vinyl tubing[g] by use of vacuum pumps into the gas sensors at the sensor manufacturer's recommended flow rate of 0.722 L/min. Samples were captured, drawn into 2 proportioned flow paths, and simultaneously analyzed with the CO_2 and oxygen sensors. Gas sampling ports were located on a horizontal plane approximately 10 inches above the floor, to approximate the breathing zone of an adult pig. The sample points were placed along a grid pattern representing the cross-section of the chamber. The gas inlet tube terminated about 3 inches above the floor at the midplane of the long side of the chamber, with inlet flow directed toward the floor. Sequential sampling across multiple sampling points introduced a 6-second time delay between the start of gas flow and the sample from a given point being read at the sensors. Data were logged in the computer program.[f]

Carbon Dioxide Generation and Delivery

For these small-scale tests, CO_2 gas was produced by heat sublimation of dry ice or by conversion of low-pressure liquid CO_2 to gas prior to each test. Dry ice was placed in open-top plastic bags, the ice-containing portion of the bag was submerged in a sealed tank of circulating hot water, and the CO_2 gas produced was directly ported into a prefabricated storage bladder.

Low-pressure liquid CO_2 exists when 2,000 kPa pressure (approx 300 psig) is applied at –17°C (0°F) and is used extensively for fire suppression systems as well as in the food service and petroleum drilling industries. Both CO_2 gas and dry ice are produced in roughly equal amounts when liquid CO_2 is released at ambient atmospheric pressure, and for some tests, headspace gas was directly ported from a liquid CO_2 delivery truck into a prefabricated storage bladder. The specific volume of gas produced from 1 kg (2.2 lb) of dry ice or liquid CO_2 is the same (19.1 cubic feet at 21°C [70°F]). As the CO_2 gas was produced, it was transferred through a 3-inch-diameter flexible hose into initially empty polyethylene storage bladders, which were fabricated on site from large sheets of black 6-mil polyethylene taped along the edges to create a seal (**Figure 4**). These containers were sized to be greater than or equal to the internal volume of each test chamber. Bladder volume was approximated by multiplying the (uninflated) base area by the typical height of the inflated bladder. The gas storage bladders permitted CO_2 gas produced from any source to be stored at near-ambient temperature prior to use (data not shown), thus eliminating potential welfare concerns associated with release of cryogenic gas or dry ice particles directly onto animals prior to loss of consciousness. The CO_2 concentration within each storage bladder was determined by monitoring the concentration as it entered the test chamber and was typically ≥ 90 vol %.

A volume of CO_2 equal to the volume of the test chamber was transferred from the storage bladder over a 5-minute period with a modified variable-speed leaf blower.[h] The inlet and outlet ports of the leaf blower were modified with 3-inch-diameter cam lock couplings to permit quick connection between the bladder and the test chamber with 3-inch-diameter flexible hoses. For test purposes, the blower fed into a custom-made venturi-manometer type flow rate sensor and the flow rate was adjusted by means of the blower throttle setting to supply 20% of the chamber volume/min (approx 150 cubic feet/min for the 30 cubic yard chamber and 75 cubic feet/min for the 14 cubic yard chamber). Gas entered the test chambers through a 3-inch internal diameter, schedule 40 PVC pipe manifold fabricated with two 90° elbows to fit over the side of the chamber under the cover and terminate 10 inches above the chamber floor with the outlet directed toward the floor. Carbon dioxide from the storage bladder was transferred into the test chamber via the leaf blower while CO_2 and oxygen concentrations were sampled and recorded by the computer program.[f] After 5 minutes, the CO_2 blower was turned off and CO_2 concentration within the chamber was monitored for an additional 15 minutes (total retention time, 20 minutes).

Figure 4—Photograph of polyethylene bladders used to temporarily store CO_2 gas (produced by sublimation of dry ice or collected as headspace gas from a low-pressure liquid CO_2 delivery truck) prior to use in swine mass depopulation experiments. Bladders were fabricated on-site from large sheets of black 6-mil polyethylene taped together along the edges to form a seal and were sized to be equal to, or larger than, the volume of the chamber into which the gas was subsequently delivered. A modified variable-speed leaf blower[h] (foreground) was used for the transfer of CO_2 gas to the chamber.

Representative CO_2 concentrations for the 30–cubic yard dumpster mock-up test chamber during the initial 5-minute wash-in period are shown (**Figure 5**). Representative CO_2 concentrations in the 14–cubic yard corral mock-up chamber are summarized for the initial 5-minute wash-in period and for the subsequent 20-minute (retention) test period (**Figure 6**). A constant CO_2 displacement rate of 20% of chamber volume/min was predicted by the computational fluid dynamic modeling and the exponential wash-in function to yield a CO_2 concentration of 63.5 vol % approximately 5 minutes after the start of gas flow.[15,22] Time to achieve 63 vol % CO_2 in the corral mock-up chamber, however, was closer to 4 minutes (a 25% displacement rate); this was likely attributable to the smaller chamber volume relative to blower speed in that test and the need for manual throttle adjustments to the blower during the 5-minute CO_2 transfer period. Concentrations of CO_2 gas > 60 vol % were retained in the smaller test chamber for 20 minutes.

On-site Field Testing

Between December 2009 and January 2011, 212 feeder pigs (offspring of Landrace X Yorkshire sows crossed to Duroc-based boars) became available for field testing of our CO_2 depopulation methods following completion of a nutrition project at North Carolina State University. These pigs could not enter the market food chain because they had received non–approved feed additives and were required to be humanely killed and disposed of at the end of the feeding trials. All work was done in accordance with animal care and use protocols approved by the North Carolina State University Animal Care and Use Committee and complied with the AVMA Guidelines for Euthanasia of Animals.[18]

The numbers of pigs for each load and chamber type used in each of 8 field tests are summarized (**Table 1**). Pigs were walked in groups from their barns along existing interbarn walkways into chambers (20– or 30–cubic yard solid waste dumpsters[i] or into 7– or 14–cubic yard temporary corrals prepared on site as previously described. Two video cameras, placed at each end of the dumpsters or at opposite corners of the temporary corrals, were used to monitor animal activity. For some tests, digital thermometers[j] were used to measure ambient temperature and chamber temperature 12 inches above the dumpster floor.

An inclined wooden panel was used to span the gap between the floor of the solid waste dumpsters and the ground to serve as a loading ramp (Figure 2); floors were covered with wood shavings to provide footing

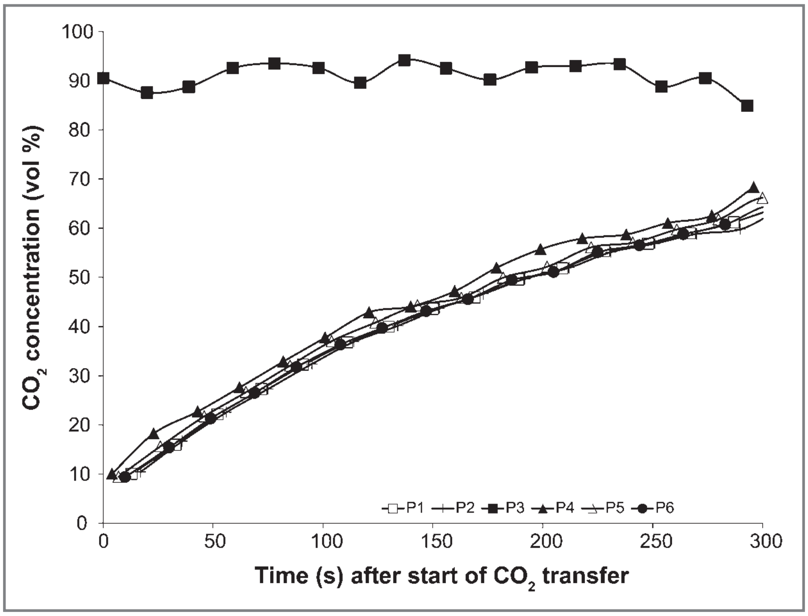

Figure 5—Concentrations of CO_2 measured in a 30–cubic yard dumpster mock-up test chamber during the 5-minute gas wash-in period. Mock-up chamber experiments were performed to refine and validate gas delivery methods prior to experiments that involved animals. Sampling ports for CO_2 (labeled P1, P2, P4, P5, and P6) were located 10 inches above the floor, at approximately the height of a pig's nose. Gas measured at the inlet (port P3) was 91 vol % CO_2 at time 0. Sequential sampling across multiple locations introduced a 6-second time delay between the start of gas flow and the sample being read at the sensor. Time 0 was the start of CO_2 delivery (rate, 150 cubic feet/min).

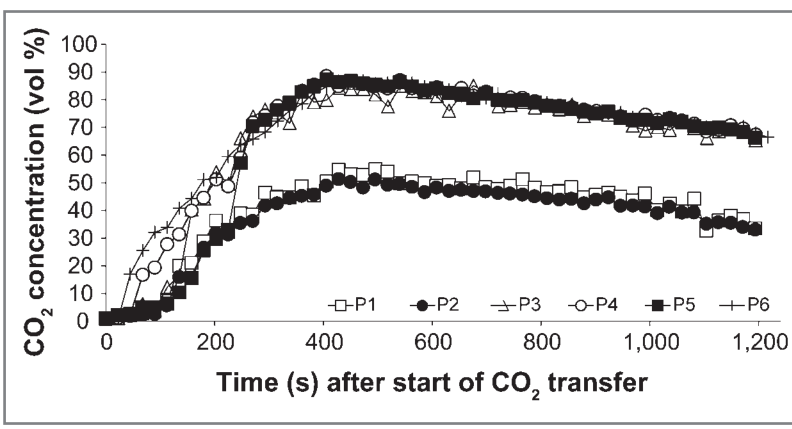

Figure 6—Concentrations of CO_2 measured in a 14–cubic yard temporary corral mock-up test chamber during the 5-minute gas wash-in and 20-minute containment period. Time 0 was the start of CO_2 delivery. Sampling ports P3 through P6 were 10 inches above the floor, whereas P1 and P2 were 24 inches above the floor. Notice that CO_2 concentrations were higher overall at the lower port locations, demonstrating stratification caused by density differences between CO_2 and ambient air. Sequential sampling across multiple points introduced a 6-second time delay between the start of gas flow and the sample being read at the sensor. In this experiment, the CO_2 displacement rate was nearly 25% of the chamber volume/min owing to manual adjustments of the blower throttle, such that the goal of 63 vol % CO_2 concentration was reached in 4, rather than 5, minutes.

for the pigs. After loading, the dumpster rear door was closed and sealed on each side and across the bottom with a polyurethane foam gap sealer.[k] Clear, 6-mil-thick polyethylene plastic sheeting was used to cover the top of the container, so that the edges draped the outside walls for approximately 20 inches, where the cover was secured with bungee cords to a wall flange at intervals of approximately 3 feet. For temporary corrals, wood shavings were also added prior to pig loading to provide additional traction. The open side panel was moved back into place after loading, the enclosure was covered with the same polyethylene sheeting, and the cover sheet and ground sheet edges were rolled together and secured with sandbags to produce a seal. Clear polyethylene sheeting was used throughout because pigs tend to naturally move from dark to light areas.[17] A

Table 1—Time to loss of righting reflex (LORR) in 212 feeder pigs (offspring of Landrace X Yorkshire sows crossed to Duroc-based boars) during 8 field tests of a gradual CO_2 displacement method for mass depopulation of swine, by date and chamber type.

				Time (s)			
Date	No. of pigs	Mean weight (kg)	Chamber type* (dimensions [feet])	Fist pig down	Many pigs (approx 50%) down	All pigs down	CO_2 source†
Dec 3, 2009	24	24.5	Dumpster (7 X 5 X 21.5)	72	87	104	Dry ice
Dec 17, 2009	48	23.7	Dumpster (7 X 5 X 21.5)	71	86	98	Dry ice
Dec 1, 2010	35	32.8	Dumpster (7 X 3.5 X 21.5)	100	127	157	Low-pressure liquid CO_2
Dec 1, 2010	6	23.7	Corral (8 X 3 X 8)	75	96	113	Low-pressure liquid CO_2
Dec 2, 2010	9	23.7	Corral (8 X 3 X 8)	70	84	102	Low-pressure liquid CO_2
Jan 6, 2010	48	21.9	Dumpster (7 X 5 X 21.5)	68	87	90	Dry ice
Jan 6, 2011	31	46.0	Corral (8 X 3 X 16)	72	83	103	Low-pressure liquid CO_2
Jan 6, 2011	11	46.0	Corral (8 X 3 X 16)	145	175	194	Low-pressure liquid CO_2

Time to LORR (evaluated as time to first pig, many pigs, and all pigs down, where time 0 was the start of CO_2 inflow) was used as a measure of time to unconsciousness.[25–28] All times were determined by consensus of 2 trained observers who reviewed video recordings obtained during experiments.

*Chambers consisted of modified enclosed commercial solid-waste dumpsters or temporary corrals that were constructed on site and enclosed on all sides.†A polyethylene storage bladder was constructed on site to contain a volume of CO_2 equal to or greater than the volume of the chamber to which gas was transferred; the CO_2 was prepared by sublimation of dry ice or by collection of headspace gas taken from a low-pressure liquid CO_2 bulk tanker and transferred to the storage bladder prior to use.

wash-out vent was cut in the top cover of each chamber as previously described.

At the start of each test, a polyethylene storage bladder (constructed on site as previously described) containing a volume of CO_2 (prepared from sublimated dry ice or low-pressure liquid CO_2 and stored for ≤ 24 hours before use) equal to or greater than the volume of the chamber was coupled to the modified leaf blower. For on-site field testing, blower speed was manually adjusted by use of the throttle to deliver the precalculated volume of CO_2 from the bladder into the chamber over a 5-minute period. Carbon dioxide gas was delivered through a 3-inch internal diameter schedule 40 PVC pipe manifold fabricated as previously described. Following the 5-minute inflow of CO_2 gas, the chambers were left undisturbed for ≥ 20 minutes. After removal of the polyethylene sheet to ventilate the chamber, all pigs were individually confirmed to be dead by 2 veterinarians (REM and WEMM) on the basis of absence of heart beat, ocular reflexes, and spontaneous breathing effort.

Video images from cameras positioned inside the dumpsters and corrals were used to estimate the onset of unconsciousness on the basis of loss of righting reflex[25–28] (LORR). The pigs were observed to move freely between the 2 overlapping fields of view provided by the video cameras. Approximately 75% of pigs were observable in each video field such that all pigs were judged to be visible between the 2 fields of view. Pigs were identified according to individual characteristics, such as color and body markings. Two trained student reviewers estimated LORR as time to first pig down (defined as laterally recumbent and unable to spontaneously right itself, or sternally recumbent and unable to hold its head up), time to many pigs down (approx 50% of all animals seen in the recordings), and time to all pigs down, by mutual agreement following post hoc review of the videos. Because the estimates of LORR were not independent observations, no attempt was made to calculate a κ coefficient.

Times to LORR, CO_2 source, and numbers of pigs observed are provided for field tests of on-site CO_2 depopulation methods (Table 1). Mean time to LORR in the first pig, many pigs, and all pigs was 84 seconds (median, 72 seconds; range, 68 to 145 seconds), 103 seconds (median, 87 seconds; range, 83 to 175 seconds), and 120 seconds (median, 104 seconds; range, 90 to 194 seconds) from the start of CO_2 delivery, respectively. Oxygen concentrations within dumpsters during the interval when LORR was occurring ranged between 13 and 15 vol % (corresponding to 20 to 30 vol % CO_2), with minimum observed oxygen concentrations between 8 and 10 vol % following completion of CO_2 inflow (50 to 63 vol % CO_2).

Temperature within the chambers quickly increased after plastic cover sheets were positioned. Representative of this, outside ambient temperature during the December 17, 2009, test was 3.3°C (38°F); temperature recorded 12 inches above the dumpster floor was 16°C (61°F) after animal loading and 31°C (87°F) 5 minutes later at the start of CO_2 inflow. Similarly, outside ambient temperature during the January 6, 2010, test was 1.6°C (35°F); temperature recorded 12 inches above the dumpster floor was 5.3°C (42°F) after animal loading and 18°C (64°F) 17 minutes later at the start of CO_2 inflow.

Discussion

Our results indicated that CO_2 gas, applied at the AVMA-recommended displacement rate of 20% of chamber volume/min for 5 minutes, can be an effective and predictable method for on-site mass depopulation of pigs. Although we used solid waste roll-off dumpsters and temporary corrals constructed on site, the method can be readily adapted, scaled, and applied to any size chamber or enclosure, including grain trailers, dump trucks, or in-ground pits, provided gas leakage along the bottom and sides of the chamber can be minimized, the available CO_2 gas volume equals or exceeds the chamber volume, and a suitable method is available to deliver the CO_2 gas into the chamber over a 5-minute period. The necessary equipment consists of common items that may be found on site or are readily available from farm supply stores and requires no special training to assemble or implement. By minimizing the number of workers required, the training of personnel is also simplified.

The field tests described in this report involved a fairly small number of animals. We were able to supply sufficient CO_2 gas for each 5-minute application by sublimating dry ice with externally applied heat or by converting low-pressure liquid CO_2 to gas and maintaining the gas in storage bladders fabricated on site prior to application. In the event of an exigent situation, methods to rapidly generate and store large quantities of CO_2 gas will be required. Near-ambient temperature CO_2 gas can be directly produced from liquid CO_2 through application of external heat; potential heat sources include geothermal sources (eg, earth tubes), air-gas cryogenic vaporizers, electric- or propane-powered industrial vaporizers, or tube-and-shell heat exchangers. In very cold weather, however, earth tubes and air-gas vaporizers will likely not be suitable for continuous gas production from low-pressure liquid CO_2, and other production or sublimation methods may be required. Fodder containers,[l] with the ends rolled and sealed, could be easily substituted for the temporary gas storage bladders described in this report. Producers and those planning to use CO_2 for emergency depopulation efforts will need to negotiate supply contracts in advance to assure access to sufficient quantities of CO_2.

The number of animals that can be processed simultaneously in a single chamber is determined by the floor area, animal loading density, and the capability to continuously provide ambient temperature CO_2 gas equivalent to 20% of the chamber volume/min for 5 minutes. Recommended transport space requirements for pigs of various weights are provided by the National Pork Board.[29] At least 40 market-weight pigs could be loaded into a 30–cubic yard dumpster at the recommended transport space of 3.98 square feet/100 kg (220 lb) of body weight. At this loading density, CO_2 use is estimated at approximately 0.64 kg (1.4 lb) of CO_2/pig[15] at a current cost of approximately $0.28/lb; this could be further optimized through computational flow dynamic modeling simulation to account for volume displacement by pigs within the chamber and by use of chambers with lower sidewalls than described in the present report, as any headspace volume above the pigs represents wasted gas. In our on-site field experiments, temperature within chambers was observed to rise quickly after placement of the polyethylene chamber cover, and temperature effects could be a welfare issue if on-site depopulation is required during hot weather. Time between loading, covering, and gas application should therefore be minimized to avoid welfare issues associated with overheating, especially during warm weather application. The use of cooling devices may be necessary in animal staging areas during such conditions.

System throughput, and therefore the time necessary to depopulate a barn or facility in an exigent situation, would vary according to pig size and numbers; available chamber type, size, and numbers; source and volume of CO_2 gas available; and the availability of workers and chamber-moving equipment.[m] Planned access to sufficient numbers of suitable chambers could greatly facilitate throughput. In 1 potential scenario, as a loaded roll-away chamber is covered and moved to the CO_2 dispensing area, an empty chamber is immediately moved into position for animal loading. Following CO_2 application, covered chambers are moved to a holding area for ≥ 20 minutes to ensure animal death, after which time the cover is removed, death is confirmed, and the chamber is relocated to a carcass disposal area where it can be emptied and prepared again for animal loading. Temporary corral chambers were readily constructed in this study and were effective in field experiments; however, effective methods for removal of carcasses afterward were not explored, and these logistics require careful consideration.

Unconsciousness is functionally defined by LORR in animals.[25–28] Individual animal LORR times were difficult to determine under the conditions of this study, because most pigs would constantly move until near unconsciousness and often move out of the video camera field of view, reducing our ability to consistently track individual pigs. Although inability to identify the time to LORR for each individual pig may have introduced a timing bias toward those who lost their righting reflex either earlier or later than most pigs, the mean time to LORR for all pigs in the 8 on-site field experiments (120 seconds; Table 1) was similar to the results of another study[24] by our group, in which neonatal pigs were administered CO_2 at 10% and 20% displacement rates with LORR ranging from 80 to 124 seconds. For comparison, facemask administration of 5 vol % of isoflurane for inhalation anesthesia of neonatal pigs produces unconsciousness within 90 seconds[30]; similar administration of 5 vol % halothane produces unconsciousness within 120 seconds.[31]

Although we believe the methods described in this report are humane on the basis of previously published research in pigs, studies specifically designed to assess behavioral responses under the conditions of administration may be needed to support that conclusion. We did not attempt to critically evaluate pig behaviors prior to LORR. Following chamber loading, pigs were observed to have what we considered typical exploring behaviors and interactions that would be expected when these animals are introduced to a new pen. Some pigs were noted to startle initially when the leaf blower used to transfer CO_2 was started but quickly appeared to become calm. Overt escape behaviors, defined as panicked running or climbing, were not observed in any animals at any time during CO_2 administration. Distress during CO_2 exposure, as identified through behavioral assessment and aversion testing, has been reported for pigs; other researchers, however, have not consistently observed this effect.[21,23,32–35] These differences may be attributable to different methods of gas exposure (eg, exposure to relatively low concentrations of CO_2 vs immersion into chambers precharged with high concentrations of the gas), variability in the strains of pigs studied, and the types of behaviors assessed. Genetic differences may have a role in CO_2 response variability. The genetic background of some pigs, especially breeds considered to be excitable such as Hampshire and German Landrace, has been reportedly associated with poor reactions to CO_2 stunning, whereas breeds such as Yorkshire or Dutch Landrace crosses were reported to have much milder reactions.[36] Duroc and Large White pigs have been observed to tolerate 30 vol % CO_2 to gain access to a food reward but to forego the reward to avoid exposure to 90 vol % CO_2, even after food is withheld for 24 hours.[21,23] However, Landrace × Large White pigs appeared to have greater aversion to a shock from an electric prod than to inhalation of 60 or 90 vol % CO_2; pigs previously exposed to 60 vol % CO_2 were willing to reenter a crate containing CO_2, whereas those previously receiving an electric shock were not.[32] Until further research is conducted, it should be considered that killing by CO_2 administration may be humane for certain genetic lines of pigs but stressful for others depending on the conditions of administration.[36]

Death must be confirmed before disposal of animal carcasses. This can be done by examination of individual animals or by adherence to validated exposure processes proven to result in death.[37] A combination of criteria is most reliable for confirming death, including lack of pulse, breathing, corneal reflex, and response to a firm toe pinch; inability to hear respiratory sounds and heartbeat by use of a stethoscope; graying of the mucous membranes; and rigor mortis.[17] None of these signs alone, except rigor mortis, confirms death. When CO_2 is administered to pigs at a displacement rate of 20% container volume/min, mean time to loss of heart beat is 8.7 minutes (range, 6 to 12 minutes).[24] Because we currently have insufficient data on the shortest CO_2 exposure time necessary to reliably ensure death in all exposed pigs, we recommend that pigs are kept in the enclosed chamber for ≥ 20 minutes after the start of CO_2 inflow under the described conditions and that death is confirmed for each animal before disposal. Any pigs that may have survived CO_2 exposure must be humanely killed using an alternate AVMA-approved method, such as captive bolt or gunshot to the head.

On the basis of results of this and other studies, the authors believe a 5-minute gradual displacement application of CO_2 to pigs at 20% of chamber volume/min, as described herein, would improve animal welfare during emergency depopulation by eliminating the need for individual animal handling and restraint; these methods would also be expected to reduce physical demands on animal workers and veterinarians engaged in depopulation. Whereas the World Organization for Animal Health currently recommends gradual displacement administration of nitrogen or inert gas–CO_2 mixtures for killing neonatal pigs for disease control purposes, it is noted that time to unconsciousness can be prolonged.[38] Unconsciousness, as determined by LORR, occurs 3 to 4 times faster in pigs administered CO_2 than in those administered a 70% nitrogen–30% CO_2 gas mixture at a similar rate.[24] The gradual displacement application of CO_2 as described in this report should be considered as one of several possible methods for mass depopulation of pigs in the event of an animal health emergency or other exigent situations.

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b. PTC Creo, PTC Inc, Lisle, Ill. Available at: www.creo.ptc.com. Accessed Jul 24, 2013.
c. FloEFD for FEP 11.3, Mentor Graphics, Wilsonville, Ore.
d. Edinburgh Instruments Ltd, Livingston, West Lothian, Scotland.
e. Model UFO-130-2G, Teledyne Analytical Instruments, City of Industry, Calif.
f. LabVIEW, National Instruments, Austin, Tex.
g. Klearon K010 (3.5 mm internal diameter; 6.4 mm outer diameter), Kuriyama of America, Schaumburg, Ill.
h. Husqvarna 125BT Power Blower, Husqvarna USA, Charlotte, NC.
i. 20 cubic yard and 30 cubic yard yd roll-off dumpsters, Elite Waste Services, Raleigh, NC.
j. Digital Dual Thermo, Control Co, Friendswood, Tex.
k. Great Stuff Big Gap Filler, Dow Chemical Co, Midland, Mich.
l. Ag-Bag, St. Nazianz, Wis.
m. Byrne H. Time and motion study and simulation application in swine transfer in pig farms (oral presentation). Inst Ind Eng Eng Lean Six Sigma Conf, Louisville, Ky, October 2012.

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