Report on the
Real-Time Exercise in the “Whole House” Carbon Dioxide Gassing Technique for the Humane Killing of Poultry in a Disease Emergency

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Executive Summary

On 16th May 2006, the Department of Agriculture and Food conducted a real-time exercise in culling a commercial poultry flock, as part of contingency planning for an avian influenza outbreak. The exercise was held near Kilmeedy, Co. Limerick in a house containing 12,000 broiler breeders, on a four-house site.

The method used was “whole house” gassing with carbon dioxide (CO₂), where the sides and doors of the house were sealed with plastic sheeting, followed by the high pressure (20 bar) infusion of liquid CO₂ until the concentration of CO₂ gas in air was sufficient to euthanase the birds throughout the house.

A total of 13.86 tonnes of liquid CO₂ was infused over 40 minutes. and was stopped when live video and audio feeds from thermal imaging and infrared cameras inside the house indicated that the birds were euthanased. The house was ventilated one hour later to remove the CO₂.

The method was 100% effective, taking approximately 40 minutes to cull all birds in all areas of the house. “Whole house” gassing with liquid CO₂ is an effective, resource efficient, rapid, welfare-friendly method of euthanasing large numbers of poultry. It has the additional bio-security benefits of keeping the birds and virus confined inside the house during the operation.

Finally and most importantly, it reduces to a minimum, the number of people in contact with live infected birds. Consequently it should be the method of choice for culling large commercial poultry flocks in a disease emergency.
Acknowledgements

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The Department of Agriculture and Food Welfare Section;
Limerick Regional Veterinary Laboratory
Central Veterinary Research Laboratory
and particularly the veterinary, technical and administrative staff of Limerick District Veterinary Office.
1 Introduction

Outbreaks of serious poultry diseases such as avian influenza (AI) and Newcastle disease (ND) are an ever-present threat. The seriousness of these diseases should not be underestimated, and is highlighted by the major epidemic in the Netherlands in 2003, where 30 million birds were culled and one veterinarian died, and the present global alert for H5N1 highly pathogenic avian influenza (HPAI). The current panzootic began in South East Asia in late 2003. To date HPAI has spread extensively within Asia, reaching Europe and Africa. Since the start of the panzootic over 220 million birds have died or been destroyed in more than 50 countries, and 247 human cases of avian influenza have been confirmed, resulting in the deaths of 144 people (W.H.O. 19 September 2006).

The protection of public health, the health and welfare of commercial and non-commercial flocks and the economic viability of the poultry industry requires early warning of disease incidents and rapid eradication of the virus. A critical component of controlling the spread of highly virulent viruses such as avian influenza is the implementation of a policy of culling as quickly as possible. This can comprise thousands of birds in multiple locations.

The combination of speed and capacity is a challenge to traditional on-farm methods of culling. In choosing a method of slaughter, the need to protect staff and other personnel by minimising the number of operatives exposed to live virus inside the house is of paramount importance. Any method of slaughter chosen must also strive to have minimal detrimental effect on the welfare of the birds.

In a cull of 30 million birds during an AI outbreak in 2003 in the Netherlands, Gerritzen et al., (2006) compared the effectiveness and capacity of different methods of slaughter and their effects on the welfare of the birds, and concluded “whole house” gassing using CO$_2$ was the preferred method of choice. With these aims in mind “whole house” gassing with CO$_2$ was selected for this real-time exercise.
The technique of “whole house” or “stable” gassing, involves liquid CO$_2$ infusion until target lethal concentrations of gaseous CO$_2$ are reached in the poultry house. Department of Agriculture and Food (DAF) observers attended successful exercises using this technique in both broiler and caged layer houses carried out in Northern Ireland in 2005. DAF subsequently conducted a successful real-time exercise in Monaghan in November 2005.

Based on the results and conclusions of these exercises, it was decided to conduct a further exercise in a larger, different type of poultry house. Following lessons learned from the previous exercises, the aim was to collect further data on effective gas infusion, CO$_2$ concentrations, temperatures, and animal welfare. The exercise was also intended to lead to practical lessons on the management of an infected site, the health and safety and bio-security issues involved, the establishment of a local bio-security centre, the preparation of a poultry house for this method of slaughter and the subsequent disposal of the birds. This exercise would also demonstrate this method to observers who would subsequently be involved in the practical application of this method of slaughter in a disease outbreak situation. Officers from Limerick District Veterinary Office (DVO) carried out the exercise with the assistance of the National Disease Control Centre (NDCC), on Tuesday 16$^{th}$ May 2006 near Kilmeedy, Co. Limerick. This location was selected, as it is a poultry dense county where familiarisation of local staff with an effective method of culling large numbers of birds during a disease epidemic is paramount.
2 Material and Methods

2.1 Poultry house

The site selected was a commercial broiler breeder site containing four houses. Three houses were empty at the time of the exercise. The fourth house (Fig. 1) was 107 m in length and 18 m in width, and contained 12,000 end-of-lay birds. The house had automatically controlled natural ventilation, with roof and side vents. There was an egg store at one end. The house was divided in two, longitudinally, by two rows of nesting boxes (1.8 m in height) in the centre of the house. Raised plastic slats at a height of 70 cm from ground level extended either side of the nest boxes, sloping downwards to a height of 30 cm. Droppings collected under the raised slats. Beyond the slats deep litter extended the length of the house. The birds were free to roam the entire house.

The exercise was divided into three phases - pre-gassing, gassing, and post-gassing. Several teams were involved in site preparation and carrying out and monitoring this exercise, all of which reported to the site co-ordinator. A bio-security centre was set up in the local area and presentations were given to observers before and after the cull.

2.2 Phase 1 Pre-gassing

2.2.1 Site assessment

A site assessment team consisting of three veterinary inspectors and two technical agricultural officers and engineers from the gas company carried out a site assessment. From previous exercises the amount of gas required to achieve a target concentration of 40% was one tonne of liquid CO$_2$ per 600 m$^3$ of house space. The area of the house was 1926 m$^2$. The highest point the birds could reach was the top of the nest boxes, which were 1.8 m in height. The house needed to be filled to a height of at least 2.5 m to ensure the birds were exposed to the gas. The minimum amount of liquid gas required for this exercise was estimated at
approximately nine tonnes. A target delivery of 13 tonnes of liquid CO$_2$ was set, to include a safety margin that would allow for loss of gas through the fabric of the house and any partly opened air vents. It is thought that losses can be in the order of 20% (Gerritzen personal communication).

2.2.2 Safety assessment and implementation

A site co-ordinator was responsible for health and safety measures on the site. A safe zone for observers was identified and cordoned off 10 m from the house. A gate officer monitored all personnel entering the site. All persons entering the site reported to the site co-ordinator who recorded the names. Instruction on appropriate personnel protective equipment (PPE) was given in advance, and all personnel on site wore appropriate PPE. Gardai Siochana (police) set up checkpoints at either end of the road and only authorised personnel were allowed to pass. The gas company operatives carried out a risk assessment prior to the exercise. Hazards were identified and protocols were drawn up to minimise risk and for use in the event of an emergency.

2.2.3 Site preparation

Site preparation commenced the day before the cull and was finalised on the day of the cull and included:

a. Liaising with an industrial gas supply company to:
   i. Supply the gas
      The liquid CO$_2$ for the exercise was supplied in a 20 tonne tanker from an industrial gas company (Fig. 2).
   ii. Construct a lance assembly
      A novel lance assembly was bolted to the concrete apron – the lance was designed by the industrial gas company to deliver gas from outside the house without the need for personnel to
enter the house to secure the equipment, and to enable the delivery of gas safely through a hole drilled in any strength wall at a range of heights (Fig. 3).

The house had walls composed of plastered 9" blocks, up to approximately 1 m height. Above this level the walls were constructed of wood and insulation. Connecting the lance to the block wall would have resulted in an internal placement of the pipe at too low a level (30-40 cm from the litter), possibly leading to dry ice and snow formation and less than optimal gas distribution, so a solution was required that would allow the lance to be fixed through the wooden part of the wall.

The lance was designed with a short stainless steel 1½" pipe with a BCGA (British Compressed Gases Association) industry standard coupling at one end for connecting to the tanker's hose. A steel sheet, approximately 45 cm² was attached perpendicularly to the pipe. A collapsible triangular assembly was designed that could be fixed to the concrete apron, and allow the lance to be fixed in place in a hole drilled in the wooden section of the wall. The lance was designed such that only a 1 x 2" hole needed to be drilled in the wall and this could be at a range of heights (15 cm to 172 cm), as the plate and lance could slide up and down the assembly. The lance took approximately 30 minutes to assemble and 30 minutes to fix the lance to the ground. In this trial the inlet lance was introduced through the timber wall at 165 cm from the ground.

b. Preparing and sealing the house

The house has a series of vents along both sides. These were sealed with 500 gauge heavy-duty builders' plastic sheeting (Fig. 4) fixed in place by nailing 2" x 1" wooden batons to the wall above and below the vents. In order to maintain normal ventilation of the birds the seals were prepared and fixed to the bottom of the vents in advance, and only fully fixed to the top of the vents immediately before gassing began. There were doors mid-way along the sides of the house and at both ends; these were also sealed with heavy plastic fixed in place with batons. The roof vents were 90% closed.
The plume of vaporising liquid CO\textsubscript{2} can extend up to 10 m and can be as cold as \(-79^\circ\text{C}\), which is the sublimation temperature of CO\textsubscript{2} at atmospheric pressure. The lance was placed at one gable end of the house pointing directly up the house. An exclusion buffer of 10 m was created with chicken wire to exclude the birds and protect them from the plume. Any loose objects or fittings in the path of the plume were secured in place. Feeders and drinkers were raised and drinkers were drained to prevent freezing damage.

c. Setting up of monitoring equipment

**CO\textsubscript{2} monitors**

Twelve sensors were placed at various points throughout the house to monitor CO\textsubscript{2} levels (See Appendix 1 – Layout of House & Table 3). In addition, the gassing company ran two plastic tubes through the walls to two external CO\textsubscript{2} monitors. The objective of the exercise was to monitor the distribution of the gas in the house and evaluate the best locations to place 1-2 sensors in normal operations to ensure that all birds would receive the target concentration of CO\textsubscript{2}. The sensors were monitored and logged remotely by running standard 3-core electric wire from the sensors to the monitoring boxes outside the house.

**Cameras**

Five infrared video cameras (three with audio) and two thermal imaging cameras (one with audio) were installed at various points within the house to monitor the behaviour of the birds throughout the exercise (See Appendix 1 – Layout of House & Table 4). The exercise was recorded fully and observers could view the cameras throughout via a large screen (Fig. 5).

**Temperatures**

Five max-min thermometers were placed in the house to monitor the min. temperature drop. Additionally seven self-contained temperature loggers were placed at designated points within
the house to measure the temperatures throughout the exercise (Appendix 1 – Layout of House).

Welfare assessment

The welfare assessment team comprised representatives from DAF Welfare Section and the Regional Veterinary Laboratory. The welfare of the birds was assessed pre-gassing. Sixteen birds were randomly selected from the front left of the house and euthanased by intra-peritoneal injection (10mls 20% w/v pentobarbital sodium: Dolethal Vetoquinol). These birds were used as controls for the post-mortem investigations.

2.3 Phase 2 Gassing

2.3.1 Health and Safety implementation

I. All personnel moved to a designated safety zone and a roll call was carried out before personnel moved to designated sites.

II. Five personal CO₂ monitors, that measure both oxygen (O₂) and up to 5% CO₂ and that give audible alerts at 0.5% and 1.5% CO₂ (when safe levels are breached), were deployed by gassing company operatives outside the house to detect any leakages and threats to personnel on site. Operatives measuring CO₂ concentrations were located at the tanker (5 m from the house), at each side of the house at a distance of 20 m, at the opposite end of the house where all the cables were running to a control room, and outside an adjacent wood chippings store where the observers were gathered.

2.3.2 Gas infusion

At 11.58 am gas infusion commenced. 13.86 tonnes of CO₂ were infused into the house for 40 minutes at full pressure. The tanker remained on site for approximately 3 hours.
CO2 concentrations inside the house were monitored from prior to the gassing until the house was ventilated.

### 2.3.3 Welfare monitoring

The temperature inside the house was monitored, and bird behaviour was monitored using cameras and audio equipment.

### 2.4 Phase 3 Post-gassing

#### 2.4.1 Ventilation of the house

Gassing finished at 12:38. At 13:17, when video camera evidence (Fig. 6) together with the cessation of noise from the house gave a clear indication that all birds were dead, gassing company operatives wearing breathing apparatus (BA) and using personal (0-5%) CO2 monitors opened the doors from the outside (Fig. 7). This was carried out while paying close attention to CO2 levels in the work areas outside the house. Portable fans that had been put in place before gassing were switched on at 13:23, and the side vents (Fig. 8) and side doors were opened between 13:32 and 13:45. The CO2 levels rapidly reduced to zero approximately five minutes after doors and vents were opened.

#### 2.4.2 Safety checks and issuing of certificate of safe entry

Industrial gas company engineers verified the clearance of CO2 from the house, by entering the house in BA and assessing CO2 concentrations using personal monitors (0-5%) measuring both CO2 and O2. On entering the house, no alarm reading at 0.5 % CO2 was triggered in the verification process. A certificate of safe entry was issued at 14:45, based on low CO2 and adequate O2 levels. Other personnel were then permitted to enter the house.
2.4.3 Welfare assessment of birds

After gassing, the distribution and posture of dead birds was assessed for evidence of panic or excessive/mass movement or convulsions (Fig. 9). Eight birds were selected randomly from each side of the house, and were examined post-mortem (see sampling locations in Appendix 1 – Layout of House). All sixteen birds were hens. Birds throughout the house were selected randomly and handled to assess whether any were frozen during gassing. Intra-cloacal temperatures were taken from nine birds at different points throughout the house.

2.4.4 Collection and disposal of dead birds and litter

Eight catchers collected the dead birds over a period of four and a half hours. Birds were transported to the rendering plant in two 40-foot lorries. For the purpose of the exercise one lorry was lined with heavy duty plastic. The litter was disposed of as per normal management practices.

3 Results

3.1 Effectiveness

3.1.1 Gassing

The gassing exercise was 100% effective. All birds in the house were euthanased. Gassing commenced at 11:58 and continued for 40 minutes.

3.1.2. Lance

The lance remained firmly fixed in place during the exercise and the metal showed no evidence of weakening or movement.
3.1.3 Sealing

Sealing of the house was adequate to allow sufficient build-up of CO₂ for euthanasia of the birds. The operatives detected no leaks of CO₂ with the personal CO₂ monitors outside the house. CO₂ gas was observed leaving the ridge vents at the end closest to the tanker.

3.2 CO₂ Concentrations

Of the 12 CO₂ sensors placed in the house, 10 remained fully functional throughout the exercise. Sensors C2 and C4 malfunctioned during the trial. Results and charts are available in Appendix 2 – CO₂ Concentrations. All sensors that operated reliably during the exercise recorded at least a 20% concentration, the concentration required to render birds unconscious. From the beginning of the rise in CO₂ concentration, the longest time taken to reach 20% CO₂ was at sensor L1, where it may have taken up to 8 minutes 50 seconds*, while the sensors closest to the infusion point (C1 & L2) reached 20% CO₂ in approximately 2½ minutes*. At the remaining sensors, it took approximately 4–6 minutes to reach 20% CO₂. Table 1 below summarises the data.

*This should not be confused with induction time, which is the time from when the birds first detect the gas to becoming unconscious.
Table 1  CO2 concentration at various time points

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Time from 0.05% CO2 to (minutes)</th>
<th>% CO2 at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20%</td>
<td>40%</td>
</tr>
<tr>
<td>R1</td>
<td>04:20</td>
<td>09:00</td>
</tr>
<tr>
<td>R2</td>
<td>04:20</td>
<td>10:30</td>
</tr>
<tr>
<td>R3</td>
<td>05:00</td>
<td>nr</td>
</tr>
<tr>
<td>C1</td>
<td>02:30</td>
<td>08:50</td>
</tr>
<tr>
<td>C3</td>
<td>04:20</td>
<td>09:00</td>
</tr>
<tr>
<td>L1</td>
<td>08:50</td>
<td>nr</td>
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<td>06:50</td>
</tr>
<tr>
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<td>04:40</td>
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</tr>
<tr>
<td>L4</td>
<td>05:50</td>
<td>14:10</td>
</tr>
<tr>
<td>L5</td>
<td>05:20</td>
<td>nr</td>
</tr>
</tbody>
</table>

3.3 Temperatures

Based on CO2 concentrations at certain sensors and the temperature logged at the nearest temperature sensor, we can estimate that the lowest temperature experienced by conscious birds was –5.8°C (Table 2 & Appendix 3 & 4). The closer the birds were to the infusion point, the higher was the risk of exposure to low temperature. There was no build up of condensation or dry ice snow, except for some very slight ice formation visible in hollows in dung build-ups close to the infusion point. No frozen birds were found. Intra-cloacal temperatures ranged from 14.2°C to 30°C.
Table 2:  Minimum temperature at CO2 concentration < 30%

<table>
<thead>
<tr>
<th>Sensor</th>
<th>20% CO₂</th>
<th>25% CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0.0</td>
<td>-3.6</td>
</tr>
<tr>
<td>R2</td>
<td>7.9</td>
<td>3.6</td>
</tr>
<tr>
<td>R3</td>
<td>20.5</td>
<td>20.0</td>
</tr>
<tr>
<td>C1</td>
<td>2.5</td>
<td>-0.5</td>
</tr>
<tr>
<td>C3</td>
<td>11.0</td>
<td>8.0</td>
</tr>
<tr>
<td>L1</td>
<td>-5.8</td>
<td>-8.9</td>
</tr>
<tr>
<td>L2</td>
<td>2.5</td>
<td>-0.5</td>
</tr>
<tr>
<td>L3</td>
<td>-3.5</td>
<td>-5.6</td>
</tr>
<tr>
<td>L4</td>
<td>8.9</td>
<td>6.2</td>
</tr>
<tr>
<td>L5</td>
<td>19.0</td>
<td>18.2</td>
</tr>
</tbody>
</table>

* note that C2 and C4 did not give reliable readings and are not included

3.4 Welfare of the birds

3.4.1 Post-mortem

External examination of the gassed birds showed no evidence of recent trauma or injury that may have resulted from either panic or distress suffered by the birds during the gassing procedure. Birds were evenly distributed throughout the house and less than 1% of the birds were found on their back. On histological examination, lesions (principally congestion and haemorrhage) were seen in the livers, kidneys and lungs of birds euthanased both by injection and by gas. There was little difference between the two sets of samples, though the lung congestion was probably more severe in the gassed birds. The changes seen were consistent with what would be expected following euthanasia, and have been described previously in laboratory animals (Feldman et al, 1976).
3.4.2 Cameras

The infrared cameras fogged up on infusion of the gas and no birds were visible on these cameras thereafter. Three infrared cameras had sound, and the microphones continued to work. At the time CO₂ levels were rising and the fog reached the birds, the level and frequency of vocalisation increased. Both thermal-imaging cameras worked throughout the exercise. Behaviour such as neck stretching, head shaking, gasping and jumping were seen as CO₂ levels increased. As the CO₂ levels continued to increase birds showed evidence of loss of posture with behaviour such as falling and rolling. Video evidence suggests that in the areas of the house that had the slowest rise in gas concentration, it took between 1 and 5 minutes from when birds first showed behavioural changes to loss of posture (an indication of unconsciousness) (Gerritzen et al. 2004).

4. Discussion

The exercise was successful and confirms “whole house” gassing as a very effective, high capacity method for the emergency slaughter of poultry. The novel lance design proved adaptable and practical to use, and reduces to a minimum, the number of people exposed to infected birds. The level of sealing carried out was adequate to achieve concentrations of CO₂ to euthanase all birds. It is estimated that a well-prepared team of three people could seal one large house in approximately three hours. Approximately nine minutes after the start of gas infusion all sensors that operated reliably during the exercise had recorded at least a 20% CO₂ concentration, the concentration required to render the birds unconscious (Gerritzen et al., 2006).

Data from the sensors indicates that the target concentration of 40% CO₂ required to euthanase poultry (Raj et al., 2006), was not recorded at three of the sensors (LI, L5 and R3). However all the birds were successfully euthanased. Possible explanations are that the birds were euthanased by prolonged exposure to lower concentrations of CO₂ (Gerritzen et al., 2004,
2006), that the environment in the house during gassing rendered the CO$_2$ sensors unreliable, or that the location of these three sensors in close proximity to solid structures i.e. secured to end walls and nest boxes, meant that the levels recorded did not represent those experienced at bird level. The sensors that recorded the lowest levels of CO$_2$ were located at the furthest distance from the gas infusion point. Due to the layout of the house, it is not unexpected that the lowest levels of CO$_2$ were recorded at these sensors.

The information from the CO$_2$ sensors, together with observed gas leakage from the roof during the exercise, and the fall in CO$_2$ levels during gassing and the “soak” period (no infusion with the shed sealed), indicate that closing the roof vents even further might result in a more predictable rise in CO$_2$ levels in the house. Closing the vents near to the infusion point may be useful to prevent leakage, and may help funnel the gas further up the house whilst closing the vents at the opposite end of the house may prevent warmer air from outside being sucked in and diluting the rising CO$_2$ concentrations. This is supported by anecdotal evidence of similar trials carried out in the UK. However closing the vents completely should be avoided, as the ambient air in the building must be allowed an escape route, thereby avoiding the creation of an air pocket where birds could survive (Gerritzen personal communication). Another measure, which might be considered, would be the use of a second tanker to infuse gas from the diagonally opposite end of the house. A further option which might be considered is to exclude the birds from these areas, represented by L1, L5 and R3, thereby ensuring their exposure to the optimum gassing conditions recorded at the other eight sensors, representing the majority of the area of the poultry house. The size of the birds and the house capacity would need to be evaluated to ensure that this doesn’t pose a welfare concern.

With the aim of continually optimising the procedure, devices are currently being developed, that would enable delivery of CO$_2$ from one tanker into the house via a number of inlet pipes (Gerritzen personal communication). These inlet pipes, which could be considered when sufficiently tested and validated, would have the added advantage of reducing the temperature
drop in the house to 10-12°C. This together with new developments in computer software aimed at predicting CO₂ gas flow in “whole house” gassing situations should provide the ability to allow a predictable and even gas distribution in the house, allowing further refinement of this procedure in the future.

Not all poultry houses will be suitable for gassing (Gerritzen et al., 2006). Key factors to consider when determining whether a house is suitable or not, would be:

- Good access for the CO₂ tanker - it must be able to get close to a wall of the house that is suitable for gas infusion
- Sealing – the house walls and floor must be capable of being sealed
- Fixing the infusion lance – there must be a solid (concrete or block) wall or a firm ground surface to attach the infusion lance to.

Containerised gassing systems are an alternative in a situation where some or all of the above criteria are not met.

Of major consideration in the process of euthanasia was the minimization of stress and discomfort to the birds. The rapid analgesic and anaesthetic properties of CO₂ are well known (Kingston et al., 2005) and this method of euthanasia of poultry is widely accepted (Welfare of Animals (Slaughter or Killing) Regulations (Wask) 1995 (HMSO 1995) and subsequent amendments, Beaver et al., 2000). It has been suggested that using certain gas mixtures (e.g. argon and nitrogen) may provide a more humane death for poultry (i.e. decreased signs of discomfort and decreased physical reaction from exposure to the gas). However as stated by Kingston et al. (2005) and Raj et al., (2006), the practicalities of this are not feasible in a large poultry house. In order to maintain a constant O₂ level of 2%-5%, the environment would need to be almost airtight. This is not possible in a large poultry house. Fluctuations in the O₂ concentrations while using a gas mixture could result in the recovery of consciousness in birds. Furthermore the practicalities of obtaining the volumes of gases required and their appropriate mixing and delivery would be extremely difficult (Raj et al., 2006).
Welfare findings during this exercise concur with published reports on “whole house” gassing with CO₂ as a welfare acceptable method of euthanasing large numbers of birds (Gerritzen et al., 2006, Kingston et al., 2005). The aim of any “whole house” gassing should be to ensure that the birds lose consciousness before they inhale the higher, more unpleasant, concentrations (>40-50% CO₂) of CO₂ (Raj et al., 2006; Gerritzen et al., 2006; McKeegan et al. 2006). The CO₂ concentrations recorded here permit induction of analgesia and unconsciousness prior to exposure to high CO₂ concentrations. Induction of hypoxia with CO₂ has been found to cause increased motor activity in birds, including wing flapping, vocalization jumping and convulsions (Gerritzen et al., 2000, 2004). Some or all of this activity was expected and was observed here, and all the behaviours observed in this exercise are consistent with those previously reported to occur during the induction process when using CO₂ in a “whole house” gassing method. In this exercise the first response seen (increase in level and frequency of vocalisation) was when the CO₂ fog reached the birds. This may be an alerting response or a sign of nervousness. The first behavioural indication of the physiological effects of CO₂ is heavy breathing, and depending on species, this may be seen at between 3% and 8% CO₂. Gerritzen et al. (in press) reported that layers noticed CO₂ concentrations at 6.6%, and behaviour changes such as head shaking were seen at 13% CO₂. Based on behavioural observations made during this exercise, the first change in bird behaviour was seen at between 5%-10% CO₂ concentration. The time between the first indication that the birds notice a changing situation (i.e. restless movements and increased alertness), and loss of posture, is approximately 1 to 5 minutes. During this induction period, deep breathing, head shaking and finally heavy gasping are observed. The duration of this induction period ranges from 1 to 3 minutes for individual birds. After this period there is a loss of posture, which indicates loss of unconsciousness. Any behaviour observed after the birds are recumbent, although aesthetically
unpleasant, is a reflex activity and therefore not perceived by the animal (Raj et al., 1990; Gerritzen et al., 2004).

Less than 1% of the birds were found on their back. Lying in this position is an indicator that birds may have had convulsions (Gerritzen et al., 2006). The distribution of dead birds in the house is an indicator of panic, fear or attempts to escape (Gerritzen et al., 2006). The even distribution of birds observed throughout the house post gassing, indicates that the reaction of the birds to the gassing was not one of fear or panic.

The data collected during the exercise, demonstrates that using the single lance method and infusing the gas at full pressure, no conscious birds were exposed to temperatures less than $-5.8^\circ C$. While this is not particularly low, and was not accompanied by post-mortem lesions indicating any cold damage to the birds, measures to continuously improve and refine this gassing technique should always be considered. Procedures, which would achieve a more even distribution of gas and therefore more mixing of the CO$_2$ with the ambient air in the poultry house, should allow for a temperature drop of no more than 10-12$^\circ$ (Gerritzen personal communication). The methods that can be considered to achieve this include those mentioned above in relation to CO$_2$ distribution.

When assessing “whole house” gassing with CO$_2$ it should be remembered that all other methods of slaughter of poultry i.e. mechanical methods, electrical methods and lethal injection require individual birds to be caught and restrained. When birds must be handled during euthanasia, welfare issues may become a concern (Raj et al., 2006). Careless handling may cause distress and discomfort (Kingston et al., 2005). “Whole house” gassing avoids the need to handle live birds. Another advantage of culling poultry in their house in a zoonotic disease situation is the minimal contact between the infected birds and personnel, which will reduce the risks to the health and safety of personnel (Raj et al., 2006) and enhance bio-security. The infected birds remain in their house, which reduces the spread of virus particles between birds and humans and potentially more widespread dispersal of the virus in the environment.
Although the CO\textsubscript{2} concentrations had not reached the target 40\% throughout the house and in some instances were falling (Appendix 2), the decision to stop gassing was based on the tonnage of CO\textsubscript{2} introduced in comparison to the amount estimated for the house volume; the CO\textsubscript{2} sensor data; video evidence that the birds were dead and microphones in the house which were not registering any bird sounds.

Site operation and management together with the health and safety measures were successful and efficient. The procedures adopted here should provide the basis for any other "whole-house" gassing situations.

5 Conclusions

The exercise conducted was successful. "Whole house" CO\textsubscript{2} gassing is considered the method of choice for the humane slaughter of poultry in commercial houses in a disease emergency, particularly where minimal contact between humans and infected birds is of paramount importance i.e. in a zoonotic disease situation. In an emergency situation it will be important to seal the house appropriately, have an accurate estimate of the amount of gas required, and to place CO\textsubscript{2} sensors in the areas where the lowest levels of CO\textsubscript{2} would be expected. CO\textsubscript{2} infusion should continue until sensors indicate that the desired concentration has been reached in all areas of the house where birds may be present.

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Appendix 1 – Layout of House

1 = Temperature Logger
L3 = Low Level CO₂ Sensor
1 = Max/Min Thermometer
L3 = Mid Level CO₂ Sensor
1 = Bird Sampling Point
1 = Infrared Camera
1 = Pipe to external CO₂ Sensor
<table>
<thead>
<tr>
<th>Sensor</th>
<th>Height from Floor</th>
<th>Distance from Left wall</th>
<th>Distance from Right wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>40 cm</td>
<td>300 cm</td>
<td>-</td>
</tr>
<tr>
<td>L2</td>
<td>45 cm</td>
<td>583 cm</td>
<td>-</td>
</tr>
<tr>
<td>L3</td>
<td>48 cm</td>
<td>583 cm</td>
<td>-</td>
</tr>
<tr>
<td>L4</td>
<td>64 cm</td>
<td>583 cm</td>
<td>-</td>
</tr>
<tr>
<td>L5</td>
<td>51 cm</td>
<td>245 cm</td>
<td>-</td>
</tr>
<tr>
<td>C1</td>
<td>164 cm</td>
<td>950 cm</td>
<td>-</td>
</tr>
<tr>
<td>C2</td>
<td>183 cm</td>
<td>950 cm</td>
<td>-</td>
</tr>
<tr>
<td>C3</td>
<td>137 cm</td>
<td>950 cm</td>
<td>-</td>
</tr>
<tr>
<td>C4</td>
<td>196 cm</td>
<td>950 cm</td>
<td>-</td>
</tr>
<tr>
<td>R1</td>
<td>52 cm</td>
<td>-</td>
<td>580 cm</td>
</tr>
<tr>
<td>R2</td>
<td>42 cm</td>
<td>-</td>
<td>580 cm</td>
</tr>
<tr>
<td>R3</td>
<td>98 cm</td>
<td>-</td>
<td>580 cm</td>
</tr>
</tbody>
</table>
Table 4  Camera Type and Heights

<table>
<thead>
<tr>
<th>Camera</th>
<th>Type</th>
<th>Audio</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Infrared</td>
<td></td>
<td>3.5m</td>
</tr>
<tr>
<td>2</td>
<td>Infrared</td>
<td></td>
<td>1.5m</td>
</tr>
<tr>
<td>3</td>
<td>Infrared</td>
<td></td>
<td>3.0m</td>
</tr>
<tr>
<td>4</td>
<td>Thermal Imaging</td>
<td></td>
<td>2.0m</td>
</tr>
<tr>
<td>5</td>
<td>Thermal Imaging</td>
<td></td>
<td>2.0m</td>
</tr>
<tr>
<td>6</td>
<td>Infrared</td>
<td></td>
<td>1.5m</td>
</tr>
<tr>
<td>7</td>
<td>Infrared</td>
<td></td>
<td>2.0m</td>
</tr>
</tbody>
</table>
Appendix 2 – CO2 Concentrations

The diagram illustrates the CO2 concentration levels during various stages of the gassing period, doors opened, fans on, and vents opened. The concentrations are represented on the y-axis, labeled as % CO2, and the x-axis represents time. Different lines on the graph correspond to different locations or conditions, labeled as L1, L2, L3, L4, L5, C1, C3, R1, R2, and R3.

The graph shows the following key stages:

- **Gassing Period**: The initial rise in CO2 concentrations as gassing begins.
- **Doors Opened**: A decrease in CO2 levels as doors are opened.
- **Fans on**: Further drops in CO2 as fans are activated.
- **Vents Opened**: Final reduction in CO2 as vents are opened.

The data suggests a significant fluctuation in CO2 levels, indicating the effectiveness of the gassing and ventilation strategies.
Appendix 3 – Temperatures recorded on max/min thermometers

<table>
<thead>
<tr>
<th>Max/Min Thermometer*</th>
<th>Before – 11:26</th>
<th></th>
<th>After – 15:15</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max °C</td>
<td>Min °C</td>
<td>Max °C</td>
<td>Min °C</td>
</tr>
<tr>
<td>1 @ R5</td>
<td>20</td>
<td>22</td>
<td>23</td>
<td>-22</td>
</tr>
<tr>
<td>2 @ L10</td>
<td>20</td>
<td>21</td>
<td>21</td>
<td>-22</td>
</tr>
<tr>
<td>3 @ L16</td>
<td>19</td>
<td>20</td>
<td>19</td>
<td>-12</td>
</tr>
<tr>
<td>4 @ R20</td>
<td>26</td>
<td>22</td>
<td>23</td>
<td>-20</td>
</tr>
<tr>
<td>5 @ L30</td>
<td>25</td>
<td>20</td>
<td>25</td>
<td>-2</td>
</tr>
</tbody>
</table>
Appendix 4  Temperatures recording on temperature loggers (see locations in Appendix 1)
References


FELDMAN, D.B., GUPTA, B.N. (1976) Histopathological changes in laboratory animals resulting from various methods of euthanasia. Laboratory Animal Science 26, 218-221


