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Killing of spent laying hens using CO_2 in poultry barns

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Abstract

In Sweden, laying hens are killed using the following methods: i) traditional slaughter; ii) on-farm with CO_2 in a mobile container combined with a grinder; or iii) with CO_2 inside the barn. The number of hens killed using the latter method has increased. During these killings a veterinarian is required to be present and report to the Swedish Board of Agriculture. Data were registered during four commercial killings and extracted from all official veterinary reports at CO_2 whole-house killings in 2008–2010. On-farm monitoring showed that temperature decreased greatly and with high variability. The time until birds became unconscious after coming into contact with the gas, based on time until loss of balance, was 3–5 min. Veterinary reports show that 1.5 million laying hens were killed, in 150 separate instances. The most common non-compliance with legislation was failure to notify the regional animal welfare authorities prior to the killings. Six out of 150 killings were defined as animal welfare failures, eg delivery of insufficient CO_2 or failure to seal buildings to achieve adequate gas concentration. Eleven were either potentially or completely unacceptable from the perspective of animal welfare. We conclude that, on the whole, the CO_2 whole-house gas killing of spent hens was carried out in accordance with the appropriate legislation. Death was achieved reliably. However, there remain several risks to animal welfare and increased knowledge would appear vital in order to limit mistakes related to miscalculations of house volume, improper sealing or premature ventilation turn-off.

Keywords: *animal welfare, carbon dioxide, killing, laying hen, on-farm, whole house*

Introduction

Normally, at the end of their laying period, spent laying hens from conventional production have no economic value, since the cost of carcase processing greatly exceeds any profit from the sale of meat. Furthermore, some end-of-lay flocks are not suitable for traditional slaughter and transport due to disease problems, poor plumage condition or poor skeletal strength. For example, Weeks *et al* (2012) found these factors significantly affected the number of hens found dead on arrival at the slaughter plant. Another problem is that journey time from the farm to the nearest slaughterhouse often exceeds the maximum time stated by national legislation. Hence, other methods, including killing of laying hens inside barns, have been developed and used as alternatives to traditional slaughter.

The welfare of hens and other types of poultry at euthanasia has been discussed and investigated by, for example, Shields and Raj (2010), for several years. On-farm killings, using CO, CO_2, whole-house killings and mobile killing devices were studied by Gerritzen *et al* (2006) during the 2003 avian influenza outbreak. The main welfare issues identified were: i) the catching and handling of birds for slaughter or killing outside the barn; and ii) the time to unconsciousness when killing birds inside the barn. Examples of whole-house methods are the introduction of agents such as hydrogen cyanide (HCN) or carbon dioxide (CO_2) gas (Berg 2009) or nitrogen (N_2) gas (A Huda, personal communication 2013) into the barns. There have also been trials investigating the use of CO_2- (Gerritzen & Sparrey 2008) and nitrogen-filled foam (Sparrey *et al* 2012; McKeegan *et al* 2013).

Behaviour and welfare

Birds from the *Gallus* family seem able to detect CO_2 at concentrations of 5.0–7.5% (Raj & Gregory 1991; Gerritzen *et al* 2007), which is lower than concentrations used for the purposes of killing. Furthermore, these birds actively avoid (Lambooij *et al* 1999; Webster & Fletcher 2004; Sandilands *et al* 2011) atmospheres with CO_2 levels above 7.5% (Raj & Gregory 1991). A recent study showed that birds provided with an opportunity to choose, avoided breathing in air with 60% CO_2 (Sandilands *et al* 2011). Despite this knowledge, CO_2 is commonly used for stunning/killing animals at slaughter plants and for emergency killings during disease outbreaks, possibly because it is nevertheless considered a 'lesser evil' when compared to other methods available.

Behavioural indicators used to estimate negative welfare in birds during euthanasia with CO_2 are head-shaking, gasping

Figure 1

A pipe (nozzle) via which CO_2 is introduced through the wall into the barn at Farm A.

Figure 2

A hose is connected from the truck carrying CO_2 to the pipe (nozzle) introducing CO_2 into the barn at Farm A.

(birds opening their beaks with the neck stretched), loss of posture and convulsions/spasms (Gerritzen *et al* 2007; McKeegan *et al* 2007; Turner *et al* 2012). Vocalisation has not been used extensively as a behavioural indicator in these studies; it was, however, used by Turner *et al* (2012). The degree of awareness during vocalisation is not known.

Legislation and commercial killing of Swedish layers

There are approximately seven million laying hens in Sweden today. These are housed in aviary systems (approximately 54%), in enriched cages (34%) and in organic and free-range systems (13%) (Swedish Board of Agriculture 2012). After the laying period, at an approximate age of 70–75 weeks, the barns are depopulated. Due to the fact that, for many years, there has only been one reasonably sized slaughter plant in the entire country accepting spent laying hens, the handling of such birds has created problems from both an economic and a welfare perspective.

Killing of animals is regulated in a specific section of the Swedish animal welfare legislation (Swedish Board of Agriculture 2008). The following is a direct translation of the article concerning euthanasia with carbon dioxide, which was in place at the point in time when these studies were carried out:

> The legislation requires that the building must be sealed before the gas is introduced. Ventilation must not be switched off until shortly before the gas is ready to be introduced. When the gas is introduced, the animals must not be hit directly by a high-pressure gas jet. Visual inspection of the animals must be possible during killing and if necessary it must be possible to measure the gas level inside the stable. The CO_2 level must reach at least 80 per cent and the CO_2 concentration must be maintained at a level of at least 60 per cent for at least 60 minutes.
>
> The planned killing shall be notified to the county administrative board or an equivalent body no later than one week in advance. The killing shall be monitored, at site, by a veterinarian from the moment the gas is introduced to the point when the barn is opened and the result can be inspected. Before the gas is introduced, the veterinarian shall approve the calculations regarding the amount of carbon dioxide to use during killing. After the killing the veterinarian shall send a completed report form to the Board of Agriculture.
>
> When new barns are built for hens and turkeys, and a pre-approval is required, a fixed pipe for the introduction of gas shall be installed.
>
> The inlets should be placed or designed so that the animals cannot be directly hit by the incoming, concentrated gas jet. Before the gas is introduced, the area closest to the gas inlet should be fenced off in such a way that no birds can be closer than ten meters from the inlet in the direction of the nozzle. Alternatively, the inlets can be aimed at an upwards angle, or a manifold can be used to swiftly distribute the gas inside the building. In order to avoid a too high over-pressure in the building, there should be openings in the uppermost part of the building so that air can escape when the gas is introduced. The amount of carbon dioxide necessary to obtain a concentration of 80 per cent can be calculated by using the formula (stable volume [m^3] × 1.8 × 0.80) (Swedish Board of Agriculture 2008).

Aim of the study

The aim of this study was to describe the technique, on a commercial basis, of using carbon dioxide in the barn for the killing of spent laying hens in Sweden. This information will assist legal authority decision-making as regards the welfare of hens during euthanasia. More specifically, the following questions were addressed:

• How does the birds' environment change during killing using carbon dioxide in the barn;

• How do the birds react and what welfare indicators might be used during on-farm killing on commercial farms; and

• Are the euthanasia events carried out in the manner intended and in accordance with legislation?

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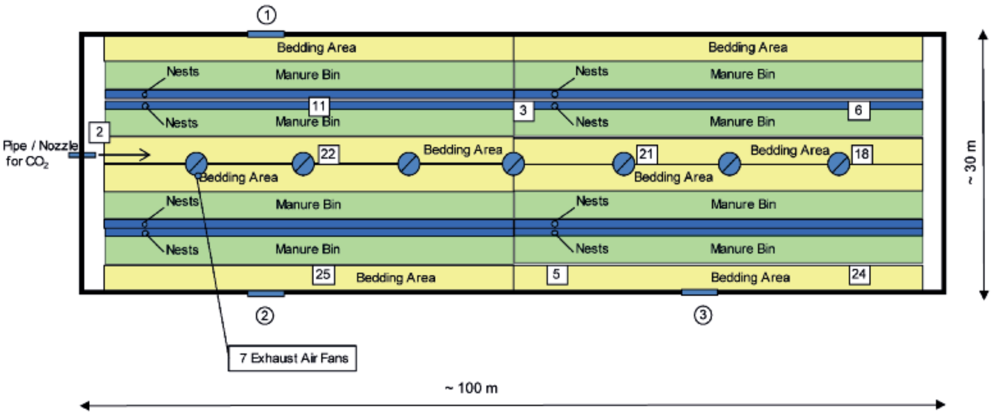
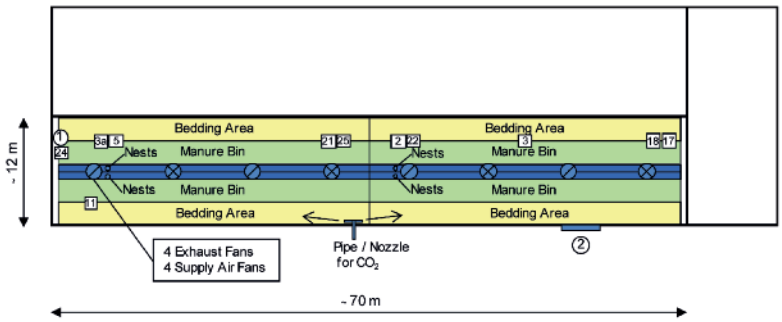
Figure 3



Layout of the studied barn at Farm A. Squares indicate temperature logger positions. Circles (1, 2 and 3) indicate inspection windows.

Figure 4



Layout of the studied department at Farm B. Squares indicate temperature logger positions. The circle (1) indicates a CO_2 and pressure measuring point and the circle (2) indicates an inspection window.

Materials and methods

Three studies were performed; one trial (Trial 1) where the technique was evaluated, and one (Trial 2) in which stunning and euthanasia effects, in relation to temperature and carbon dioxide concentration, were studied. Finally, the official veterinary reports from killings were analysed (Trial 3). All farms in Trials 1 and 2 had loose-housing systems. In the record analysis, however, barn systems with both aviaries and furnished cages were included. The decisions to kill the flocks in this study using CO_2 were taken by the egg producers (or, in a number of instances, by the authorities in disease control situations), and killings were carried out under the full responsibility of the producers, without any interference from the research team as to the procedures or actions taken. Figures 1 and 2 illustrate how the CO_2 was delivered and administered into the poultry barns.

Trial 1: Technical evaluation

Data were collected from two different farms (A and B). Farm A was a single-tier, loose-housing system with litter area, manure pit, perches and nests and the barn studied contained 22,000 birds (Figure 3). Farm B was similar but the section of barn studied was smaller and housed 8,000 birds (Figure 4). Both farms utilised under-pressurise ventilation systems with roof-mounted exhaust fans and outdoor air sucked into the barn from above the roof through evenly distributed ducts and inlets along the full length of the barns. Birds killed during the study were of the hybrid Bovans white. Temperature was measured using Tinytag TGP data loggers (Gemini Data Loggers, Chichester, UK), at ten and eleven locations in the barns for A and B, respectively. Carbon dioxide concentration at Farm B was calculated from measured oxygen (O_2) concentration. The O_2 concentration was measured using a Lutron

Figure 5

Positioning of the floor-level video camera at Farm C.

Figure 6

Positioning of the video camera at the upper level, Farm C.

DO-5510 instrument (Lutron Electronic Enterprise Co Ltd, Taiwan) placed as far away as possible from the CO_2 inlet.

There was no available second functioning device for these measurements and O_2 was measured at just one spot at a height of 1.0 m above floor level. According to the manufacturer, resolution and accuracy were 0.1% O_2 and ± 0.7% O_2, respectively. For the calculated CO_2 concentration this gave a resolution of 0.5% and an error no worse than approximately ± 3%. At the start of the gassing, the calculated CO_2 concentration (0%) was in accordance with expected values for a barn (500–2,500 ppm; 0.05–0.25% CO_2) indicating no such large error.

At Farm A, all air inlets were sealed before introducing CO_2, and openings through manure culverts and egg conveyors were also sealed while the exhaust fans were turned off a couple of minutes (< 5) before gassing, without closing dampers in the openings. At Farm B, openings through manure culverts and around egg conveyors etc were thoroughly sealed, the fans were turned off about 3 min before gassing, at which point the dampers were closed leaving only small free areas around the closed dampers open.

The principal layout of the studied barn sections at Farms A and B are shown, respectively, in Figures 3 and 4, with the loggers' positions indicated.

Carbon dioxide was introduced into the barns at a gas flow rate of 375 kg min^{-1} at Farm A and 540 kg min^{-1} at Farm B, corresponding to approximate 'air' exchange rates per hour of, respectively, 1.4 and 6.0. At Farm A it took approximately 18 min to deliver the total calculated amount of CO_2 into the barn while only taking 6–7 min at Farm B. At Farm B the over-pressure inside the barn was registered during gassing using a portable manometer (EMA 84, Halstrup Walcher GmbH, Schwartzwald, Germany) connected to a Tinytag TGPR-0704 data logger (Gemini Data loggers, Chichester, UK) via a voltage output.

Trial 2: Behaviour of the birds during killing in relation to temperature and carbon dioxide concentration

Two Farms (C and D) were chosen for data collection of bird behaviour. Both were aviary systems, housing, respectively, 13,600 and 11,500 Bovans white, hybrid birds. The ventilation system at Farm C's studied house was an under-pressure system with roof-mounted exhaust fans and outdoor air sucked into the barn though the roof via ducts and inlets evenly distributed along the full length of the barn. For Farm D there was an under-pressure ventilation system with wall-mounted exhaust fans and ceiling inlets for outdoor air placed in two rows along the full length of the barn.

At Farm C, the egg belt and manure system etc were not sealed prior to gas distribution, whereas at Farm D these potential gas escape routes were sealed to prevent leakage.

The CO_2 concentration was calculated from oxygen (O_2) concentration measurements. Two portable OxyGuard handy atmosphere sensors (OxyGuard international A/S, Denmark) were used to measure O_2 concentration. The typical accuracy of these devices were given as ± 1% of measured O_2 concentration ± 1 digit (0.1%) which, in the worst case scenario, will produce an error of about ± 1.5% for the calculated CO_2 concentration. At Farm C, two Tinytag miniloggers were used for temperature measurement and the O_2 sensors were read manually outside the building at intervals of 60 s. This started when the gas was turned on and ended 35 min after the gas was turned off. This compared with Farm D where loggers were programmed to start 20 min before the planned onset of gas distribution into the house and measured at 30-s intervals.

Events were video-recorded with cameras placed alongside oxygen sensors (Figures 5 and 6). One Sony digital camera (model DCR-PC6E, Sony, Tokyo, Japan) and one Panasonic digital camera (model NV-DS37EG M, Panasonic, Osaka, Japan) were used to record both high above the floor and at a low level. Cameras began recording a couple of minutes prior to the introduction of gas (see Figures 5 and 6 for exact positioning of cameras).

Animal behaviour was recorded from the onset of gas introduction until movement and/or vocalisation had ceased or further observation became impossible as a result of fog generation (Table 1). When analysing the videotapes, birds' behaviour was recorded every 5 s via scan sampling of all birds visible. Fog accumulation limited the number of birds clearly visible to between 0–10 birds. Behaviours were classified into one of four categories: i) animals apparently starting to sense changes in the atmosphere; ii) breathing difficulty starts; iii) animals losing posture; and iv) animals completely lose posture and become unconscious.

Three birds from Farm C and two from Farm D were sent for post mortem giving a total of five birds. These examinations covered gross general pathology, including signs of disease prior to killing, but also indications of lesions related specifically to the killing process, such as frost lesions. Two birds from each site were picked out arbitrarily from where the video recordings were made a couple of hours after gassing (once barns were safe to re-enter). And, from Farm C, one hen was picked from the area closest to the gas inlet.

Trial 3: Reports from inspecting veterinarians

National legislation dictates that euthanasia with CO_2 on farm sites should be inspected by a veterinarian and the event reported to the Swedish Board of Agriculture. We requested copies of every veterinary report from the Swedish Board of Agriculture for killings dating from 2008–2010. These reports contained a myriad of information concerning the events: where and when the killing was carried out; how many birds were killed; the amount of CO_2 used; the barn volume; if and how the birds were kept away from encroaching in to the vicinity of the gas inlet; the time when ventilation was turned off prior to killing; how long after the start of gas distribution vocalisations could be heard; how long after the start of gas introduction was the last sign of life (ie breathing movement, other movement or vocalisations); if the birds were in an acceptable condition prior to the killing; if the animal welfare inspector was duly informed; if the gas delivery company had provided instructions to the farmer beforehand; and also room for comments. The reports were summarised to evaluate how the procedure was working. Only descriptive statistics were used and figures drawn in Microsoft Excel®.

Table 1 Behavioural registration at Farms C and D.

Behaviour	Description
Category 1	
Beak opens and closes	The bird opens and closes the beak. Has been described as 'tasting behaviour' (Gerritzen *et al* 2007; Coenen *et al* 2009)
Head-shaking	The bird shakes its head
Category 2	
Gasping	Breathing with beak open and stretched neck
Category 3	
Loss of posture	Standing birds observed primarily sitting down, while perching birds reel and use their wings to maintain balance
Loss of neck tonus	Perching birds, lower their heads, often below perch level
Category 4	
Loss of posture	Sitting birds lay their heads down and/or fall over on to their side or back. Birds on a perch lose their grip completely and fall
Convulsions/spasms	Sudden wing or body movements
Vocalisation	The point at which vocalisation ceased was noted as well as the degree of vocalisation during the phase when gas enters the house
Movement ceased	No movement detected

Results

Trial 1: Technical evaluation

Temperatures varied substantially throughout different parts of the barn (Figures 7, 8 and 9). A rapid decrease was observed when carbon dioxide was let in. The lowest temperature loggers could register was –30°C and this was observed close to the gas inlet as well as further away from the gas inlet above the nests (logger 3), which indicates air also mixing vertically throughout at least half of the barn (ie 50 m away from the gas inlet).

Further from the gas inlet the temperatures decreased less and slower. An intermediate temperature decrease could be seen in the central part of the barn.

Gas mixing with the air in the barn had a profound effect on the temperature profile. The time taken for temperature reduction to become apparent varied for different parts of the barn and with distance from the gas inlet. At Farm A, this varied from 0–6 min after the start of the gas distribution with temperatures affected more quickly close to the gas inlet and a 6-min delay in the onset of temperature decrease registered furthest away from the gas inlet. At Farm B, temperature was

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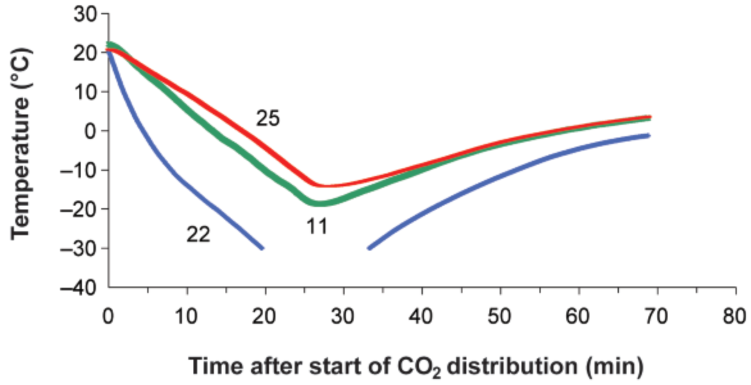
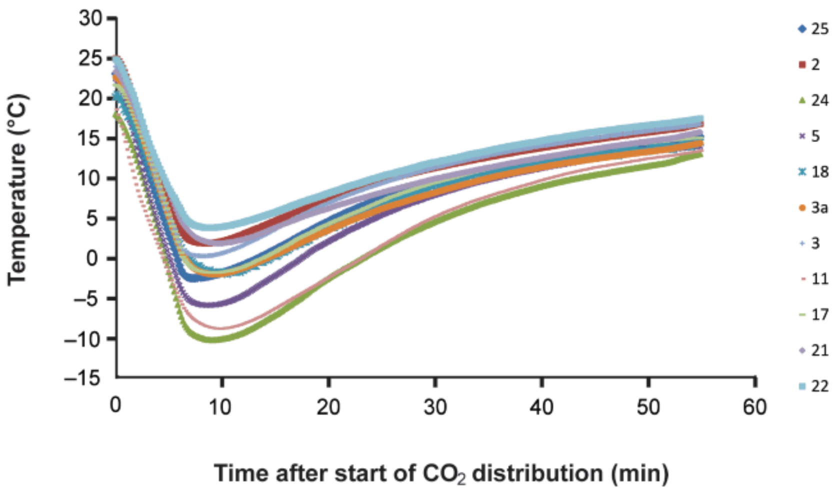
Figure 7



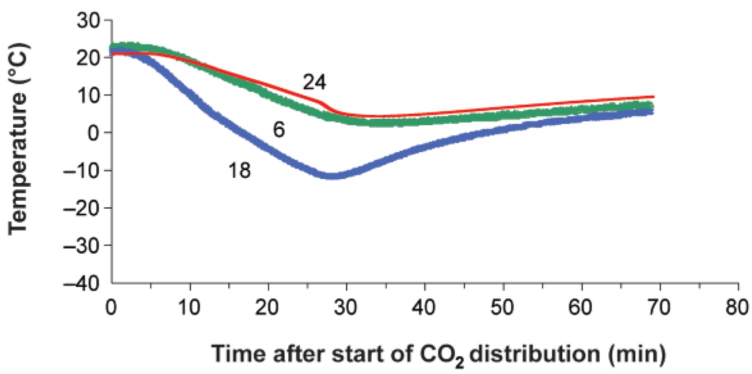
Temperature development approximately 25 m from the gas inlet at Farm A plotted against time after start of gas distribution (line numbers refer to logger positions; see Figure 3 for logger positions).

Figure 8



Temperature development at Farm B plotted against time after start of gas distribution (numbers on the right-hand side refer to logger positions; see Figure 4 for logger positions).

Figure 9



Temperature development at the end of the barn opposite the gas inlet at Farm A (about 90 m from the inlet), plotted against time from start of gas distribution (line numbers refer to logger positions; see Figure 3 for logger positions).

affected between 0 and 0.5 min after the onset of gas distribution and no clear pattern could be seen due to the distance between the gas inlet and where the loggers were placed.

The CO_2 concentration, as calculated from the O_2 measurements, inside the barn at Farm B and at a distance from the gas inlet increased to 50% approximately 5 min after start of CO_2 distribution, reaching 60% and above after 8 min.

There were no signs of high over-pressure inside the building during gas introduction at Farm A with larger openings, while some cracking sounds and bulging of doors was observed at Farm B, where only small openings were left open during gas introduction. The over-pressure at Farm B increased up to a maximum of 700-800 Pa when all the CO_2 was delivered to the barn and thereafter it decreased to zero within a couple of minutes after the peak.

Table 2 Time (min: s) after onset of gas distribution for observed specific bird behaviours at Farm C related to the effect of carbon dioxide. Gas distribution continued for 14 min.

Behaviour	Low level in the system			High level in the system		
	1st bird shows the behaviour	~ 50% of the birds show the behaviour	Interval when behaviour is seen	1st bird shows the behaviour	~ 50% of the birds show the behaviour	Interval when behaviour is seen
Beak opens/closes	01: 10	X	X	01: 30	X	X
Head-shaking	01: 10	X	01: 10–03: 10[†]	01: 50	X	01: 50–04: 00[†]
Gasping	01: 05	01: 50	01: 05–04: 10[‡]	01: 35	02: 00	01: 35–05: 30[‡§]
Loss of balance	02: 05	X	X	X	X	02: 00–04: 00
Reduced neck tonus	03: 10	X	X	04: 00	X	X
Complete loss of balance	02: 50	03: 10–04: 10[‡]	X	04: 10–04: 40	04: 40–05: 00	X
Spasms/forceful wing flapping in lying birds	X	X	X	X	X	05: 00–07: 00[¶]
Last movements seen		04: 10[‡]			09: 00–11. 00[¥] (breathing) 15: 00[¤] 16: 00–18: 00[#]	
Vocalisation level	Intensified after onset of gas distribution, decreasing from 05: 00[¶]			Intensified after onset of gas distribution, decreasing from 05: 00[#¶]		
Fog disturbing observations		03: 10–04: 00		05:00–06: 00, 09: 00–14: 00, 15: 00–16: 00		
Complete fog		04: 00–15: 10			05: 30–09: 00, 14: 00–15: 00	

X indicates missing information due to fog. [†] Turns into forceful neck movements where the neck is stretched and the birds are gasping: some lose their balance; [‡] Fog; [§] Obvious gasping with elevated head seen also in lying birds; [#] One bird at 15 min 20 s; [¶] Few birds vocalised between 7–15 min; [¥] At least 2 birds; [¤] At least 4 birds (wing movements).

Table 3 Time (min: s) after onset of gas distribution for observed specific bird behaviours at Farm D related to the effect of carbon dioxide. Gas distribution continued for more than 9 min.

Behaviour	Low level in the system			High level in the system		
	1st bird shows the behaviour	~ 50% of the birds show the behaviour	Interval when behaviour is seen	1st bird shows the behaviour	~ 50% of the birds show the behaviour	Interval when behaviour is seen
Beak opens/closes				02: 05	X	X
Head-shaking				02: 05	X	02: 05–03: 30[†]
Gasping		No observations due to fog		02: 05	02: 15	02: 05–03: 30[†]
Loss of balance				02: 30	X	X
Reduced neck tonus					Not observed	
Complete loss of balance				03: 15	03: 30	03: 15–04: 00[†‡]
Spasms/forceful wing flapping	Forceful flapping can be heard, particularly during a period with increased vocalisation 05: 40–08: 50			Forceful flapping can be heard, particularly during a period with increased vocalisation 05: 00–08: 45		
Vocalisation level	Intensified after onset of gas inlet, decreasing from 04: 00. Again, increasing 05: 40 and decreasing 08: 50. Last vocalisation heard 11: 30			Intensified after onset of gas inlet, decreasing from 04: 00. Again, increasing 05: 00 and decreasing 08: 45. Last vocalisation heard 10: 50		
Fog disturbing observations		00: 40–00: 45			02: 00–03: 30, 10: 00–11: 00	
Complete fog		00: 45–26: 00			03: 30–10: 00	

X indicates missing information due to fog. [†] Fog obstructs visibility; [‡] All visible birds show the behaviour.

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Trial 2: Behaviour of the birds during killing in relation to temperature and carbon dioxide concentration

Behaviour of the birds

As gas began being distributed the birds moved quickly away from the gas fog, in particular at the lower levels in the system. After approximately 50 s the birds moved less and stood more. An increase in vocalisation was registered from the gas inlet area immediately after the onset of gas distribution. Tables 2 and 3 show results from Farms C and D; a summary of recorded behaviours (5-s interval scan sampling), and times given for when the first bird and 50% of the birds showed a particular behaviour. Durations are shown where relevant. At times, fog made visual observations difficult or impossible and this is indicated in the tables. At Farm D, only animals perching at the upper level were able to be observed and then only until they lost balance and fell to the lower level.

Birds at the lower level in the system showed signs of being affected by the gas, and gasping along with beaks opening and closing was first registered after approximately 2 min. Animals at the higher levels took somewhat longer. The hens started to display problems with balance, simultaneously, both high and low levels, approximately 2 min after the commencement of gas distribution. After 3 min some birds, at low levels in the system, had difficulty holding their heads upright. Higher up this phenomenon took 4 min to be observed. This trend was also observed for the time taken for birds to lose their balance and either fall on their side or off the perches, with an approximate 1-min delay between lower and upper levels (3 and 4 min, respectively). After 4–5 min observations became harder to register due to fog. Breathing and other movements could however be observed. Breathing was seen for 11 min after the onset of gas distribution, and wing flapping persisted in a few individuals up until the 18th minute. It can be assumed that irregular, shallow breathing may have occurred in these latter individuals although we were not able to detect this. Gasping was seen until the fog was thick. At the high level it could be observed clearly for 5.5 min. Vocalisation became intensified from the first signs of the birds being affected by the gas until approximately 5 min after the onset of gas distribution. Some birds were heard vocalising until 16 min after the onset of gas distribution.

Breathing was seen in a few birds until the 11th min (around 38% CO_2) and the last movements were observed after 18 min (around 48% CO_2). However, the oxygen probe was not necessarily located in the exact vicinity of the birds in question and hence it is quite possible that the CO_2 concentration where these birds were located was substantially lower at that point in time. At Farm C, the vocalisations decreased after the 5th minute (around 17% CO_2) and the last vocalisation was heard at 15.5 min (corresponding to 49% CO_2). At Farm D, vocalisation was most intense between the 5th–9th min (corresponding to 30–46% CO_2). The last vocalisation at Farm D was heard after 11.5 min at 48% CO_2.

After killing, the animals lay spread out evenly in the centre of the barn and on the upper levels. On the floor in front of the gas inlet the litter had been blown away and a small number of birds lay there.

Environment within the barns during killing

At Farm C, the lowest temperature at floor level, –3.2°C, was registered after 13 min, in the centre of the system and, at the high level, temperature reached 10.0°C after 19 min. For Farm D the corresponding temperatures and times were –3.2°C after 15.5 min at the low level and 11.2°C after 28 min at the high level.

At the barn at Farm C the CO_2 concentration at the higher level increased continuously until a peak of 49% was attained 15 min after onset of gas distribution and thereafter, the concentration decreased (Figure 10). A value of 8.6% was recorded 35 min after start of the gas introduction. A similar pattern was seen at Farm D and the higher level CO_2 concentration reached a peak of 48% after 13 min, and then decreased to 10.0% after 35 min (Figure 11). At both farms, the observed concentrations of CO_2 at floor level were somewhat higher than at the higher level, at times when both monitoring devices were functioning (8–10 min).

As early as the second minute, affected breathing was seen in birds, corresponding to a CO_2 concentration of less than 5%. The birds had problems maintaining their balance at concentrations above approximately 5% CO_2 and lost their balance completely at 13–17% CO_2.

Post mortem examination of birds

Results showed a ruptured liver in one bird and mild catarrhal inflammation in the trachea was seen in two birds. No birds showed any signs of frostbite and no other histological signs related to the CO_2 killings were found.

Trial 3: Record analysis, reports from inspecting veterinarians

According to reports, 590,900 hens were killed using CO_2 in 49 separate instances in 2008, 396,306 hens in 47 instances in 2009, and 563,138 at 54 in 2010.

Out of the total of 150 instances of euthanasia we define six (one in 2008, two in 2009 and three in 2010) as failing in terms of animal welfare. These failures included hens in a completely different section of the building section almost being killed by mistake. There was a failure to seal the building to achieve adequate concentration causing a substantial extension of the time taken to kill the hens, ventilation was turned off several hours before gas entry and, on one occasion, an insufficient volume of gas was delivered. Another eleven out of these 150 killing events were perilously close to impinging upon animal welfare due to ventilation being turned off for 20 min or longer prior to gas injection or through insufficient sealing of the building leading to large gas leakages (Table 4).

In the reports it was rare to encounter any information on the time from onset of gas distribution until cessation of vocalisation or the last signs of life. Of the 150 euthanasia events, only 52 supplied information about bird vocalisations. On average, vocalisation was heard until 8.8 min after the onset of gas distribution. Only ten out of the 150 reports provided any information on the last signs of life. These ten observa-

Figure 10

The CO_2 concentration at floor level (blue) and the higher level (red) at Farm C in relation to the time from the start of gas distribution.

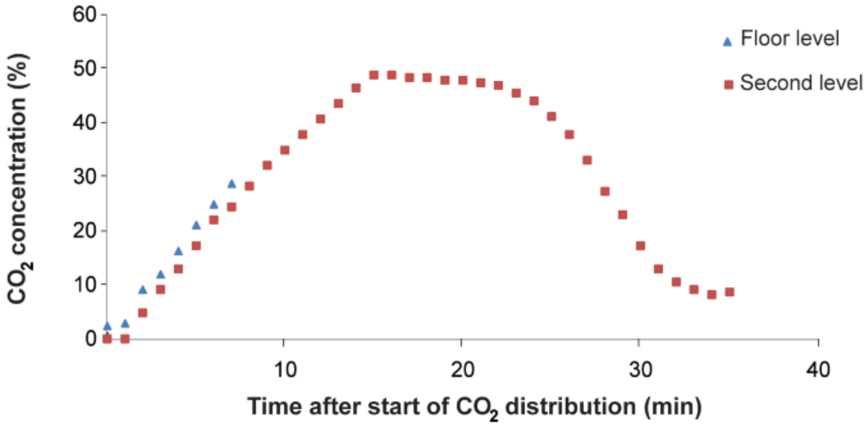

Figure 11

The CO_2 concentration at floor level (triangles) and the higher level (squares) at Farm D in relation to the time after the start of gas distribution.

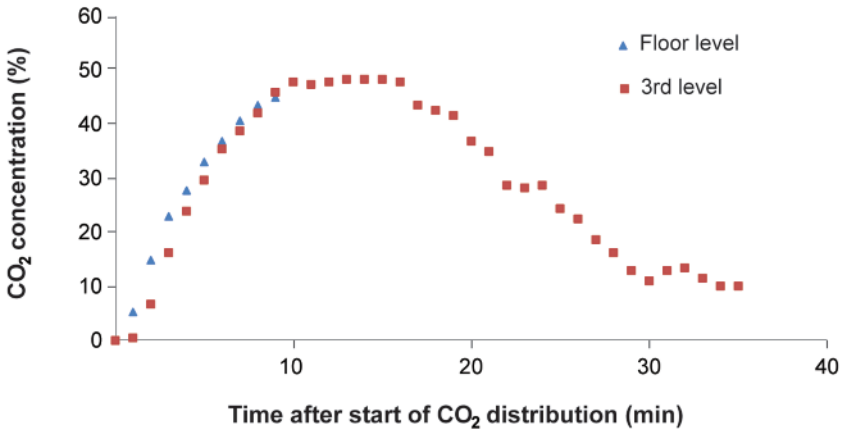

Table 4 A list of killings of end-of-lay hens during 2008, 2009 and 2010 which can be considered as failures or as impacting upon animal welfare, including identification of the problems encountered.

Year	Failures	Incidents
2008	• Insufficient sealing: a number of birds in adjacent compartment died	• The calculated amount of gas was insufficient • A door flew open as a result of over-pressure • Ventilation turned off 20 min in advance
2009	• Insufficient amount of gas delivered, entire procedure had to be repeated • Ventilation turned off 2 h in advance	• Ventilation turned off 20 min in advance • Ventilation turned off 1 h in advance
2010	• Two occasions where insufficient gas was delivered, entire procedure had to be repeated • Ventilation turned off 3 h in advance	• Five occasions where ventilation was turned off 20 min to 1 h in advance • A hatch flew up, gas leaked, hatch was closed quickly and surplus gas added immediately

tions gave an average of 11.2 min between the commencements of gas delivery and the last sign of life in the barn.

Although recording and reporting it, none of the veterinarians ever wrote specific comments on the time of turning off ventilation, with it sometimes being up to 3 h before the gas was turned on. Any incidents of gas leakage were alleviated by the gas delivery company, as they are routinely in possession of more than the ordered amount of gas.

Another common non-compliance in relation to the legislation was that farmers did not inform the official animal welfare inspectors prior to euthanasia. This happened in 30% of the killings in 2008, 28% in 2009 and 24% in 2010. The presence of animal welfare inspectors is, in direct contrast to veterinarians, not required, and no information exists as to whether the animal welfare inspectors were present or not during the killings that they had been informed about.

Discussion

There are two aspects of animal welfare we should be aware of concerning the use of CO_2 in the barn to euthanase spent laying hens. Firstly, there are the welfare aspects of the method of killing itself, which have been studied extensively (eg Sandilands *et al* 2011; McKeegan *et al* 2012; Turner *et al* 2012). Secondly, we have the hazard to welfare when the method is performed on a larger scale, commercially, and the legislation or standard operating procedures for the euthanasia are not adhered to. This study shows that there are grounds for concern regarding the latter.

Technical evaluation

Trial 1 showed that the forceful distribution of CO_2 into poultry barns of differing designs leads to variable degrees of mixing of the gas with air in the barn. The temperature decreases rapidly, more so close to the gas inlet. A division in the air with cold gas 'floating' along the floor appeared to occur furthest away from the gas inlet. At Farm A, as long as 6 min elapsed after the onset of gas distribution before the temperature was affected at the rear end of the barn, indicating that CO_2 had taken at least this length of time to reach the birds in this part of the barn. However, it is possible that low but detectable levels of CO_2 may have reached the birds prior to temperature being affected, and it is hard to say whether or not this time delay negatively impacts on the welfare of birds in this part of the barn.

We found that the temperature during a CO_2 killing could drop below –30°C (Farm A). Typically, the closer to the gas inlet, the more the temperature dropped. No birds were sent for post mortem examination from this farm, but no signs of frost-related lesions were found in any of the hens sent for post mortem examination from the other farms in this study. McKeegan *et al* (2012) found that the house temperature did drop below zero (minimum recorded value was –13°C in close vicinity to a bird) but saw no evidence of birds physiologically freezing. Turner *et al* (2012) recorded a minimum barn temperature of –23°C and a hen body temperature of 38.3°C at the estimated time of loss of consciousness in their study bird.

Even though calculations of the amount of CO_2 were designed with the aim of reaching a concentration of 80% in the house this was, as expected, never achieved, as some leakage is inevitable even in reasonably well-sealed buildings. This indicates that the equation used commercially is not sufficient if the aim is truly to reach an 80% CO_2 concentration throughout the entirety of the building. However, in all killings, where gas was provided according to the calculations, death was achieved reliably. This is to be expected, as CO_2 is lethal at concentrations far lower than 80% (Raj *et al* 2006; Sparks *et al* 2010; Gerritzen *et al* 2012). Furthermore, as the gas is heavy the concentration will be expected to be highest at floor level, where many birds are located. However, in housing systems where birds are found at higher levels (multi-tier aviary systems and three-tier furnished cage systems) death was readily achieved, indicating that the legislated CO_2 concentration and normal leakage of gas was sufficient to kill the birds.

During killing with CO_2 gas in commercial barns, the openings between the barns and the exterior must be reasonably large since a considerable amount of gas is delivered in a limited amount of time. When gas enters the barn, roughly the same amount of air must leave the building. Barns being very tight with only small openings to the outside during the CO_2 gas-filling procedure are a potential threat to animal welfare. If high over-pressure is reached, doors or windows can be blown open leading to a delay in killing due to substantial gas leakage, time to restore the building, and time for the barn to re-fill with CO_2. This risk was identified at Farm B where very high pressure was achieved, and in one of the failure (incident) reports from killings, where a door was blown open. Furthermore, if pressure is too high, ambient air may be trapped in 'air bubbles' within which animals may survive.

Bird behaviour

Birds vocalised and showed breathing movements up to 15 and 18 min, respectively, after the onset of gas distribution. There are two recent detailed studies of killing birds with aerial CO_2; McKeegan *et al* (2012) who studied caged laying hens and Turner *et al* (2012) studying one flock of caged birds and one with loose-housed birds. Both have investigated, in detail, physiological and behavioural parameters during euthanasia using birds fitted with equipment for measuring ECG and EEG, as well as body temperature (McKeegan *et al* 2012; Turner *et al* 2012). Breathing behaviour along with ECG and EEG suggested that the birds were aware of the CO_2 for, on average, 4–8 min prior to losing consciousness and the concentration of CO_2 being around 20% (McKeegan *et al* 2012). Unconsciousness in connection with loss of posture has been studied by Gerritzen *et al* (2004) and Benson *et al* (2012), who found that loss of posture may be used as an indicator for unconsciousness, at least in broilers. The average time to death for the birds fitted with equipment to measure EEG was 5 (Turner *et al* 2012) and 16 min (McKeegan *et al* 2012) from the start of gas distribution. However, in the McKeegan *et al* (2012) study movements were registered from birds in the flock until 22 min after onset of gas distribution, which is in accordance with the times recorded in this study where the last movements were seen 18 min after onset at Farm C. Although birds that move are not dead, their level of consciousness is not known here as well for many of the birds in the studies by McKeegan *et al* (2012) and Turner *et al* (2012).

As the barns are large and the spreading pattern of CO_2 varies with time, the behaviour of birds in different parts of the barn will vary. Moreover, the technical design and procedures used will affect the time for a certain exposure and its development over time throughout different parts of the barn. The gas inlets in our study were placed approximately 0.7 m above the floor, ie at roughly bird-height from the ground, whereas in the study by McKeegan *et al* (2012) the gas inlet was placed in the manure pit, some 3 m beneath the birds. In the study described by Turner *et al* (2012) a specially designed device to vaporise the liquid CO_2 was

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deployed and this was placed on the barn floor. Furthermore, the time to reach a lethal concentration of carbon dioxide varied substantially between the studies. In the McKeegan *et al* (2012) study, 60% carbon dioxide was reached in approximately 30 min, whereas in the present study about 50% was reached within roughly 10 and 15 min at Farms C and D, respectively (and 60% was reached in about 8 min at Farm B). Turner *et al* (2012) performed two experiments, one in a cage barn where CO_2 concentration exceeded 60% after 7 min and one in a loose-housing barn where a maximum of 47% was reached after 32 min. In our study, a rapid inlet of CO_2 was correlated with a rapid decrease in temperature, however there are no indications that this drop in temperature severely affected the welfare of the birds.

Risks for animal welfare during whole-house killings

A small-scale study by McKeegan *et al* (2013) tested gas-filled foam as a euthanasia method. Hens and broilers were submerged in foam containing CO_2 or N_2 and physiological and behavioural parameters were studied. In this case, behaviours indicating aversiveness were observed shortly before submersion in the foam when it contained CO_2 but not until submersion in the case of nitrogen. Unconsciousness occurred within 30 s and death of the birds was achieved reliably.

A pilot study (Gerritzen & Sparrey 2008) investigated the animal welfare aspects of using a combination of foam and carbon dioxide. The advantage of this combination is that the building does not have to be sealed as the gas is contained within the foam bubbles. The animal welfare implications were concluded to be milder than those seen in large-scale cold CO_2 killing. However, more studies are needed to ascertain whether the effects are also milder on a larger scale and if this method is practically applicable for laying hens in various housing systems.

When it comes to the practical use of CO_2 in barns we found that veterinarians inspecting the farms filled in the Swedish Board of Agriculture forms in a way that indicated that they did not fully understand their purpose, which suggests the forms could be improved. One factor that is clearly important for the welfare of the birds is the time-span between turning off ventilation and the onset of gas distribution. In many cases the time of day is noted, without noting when gas distribution begins, which renders this information completely irrelevant from the perspective of animal welfare. If an HACCP (Hazard Analysis Critical Control Point) approach was to be used for this method of euthanasia, the time interval between ventilation turn-off and gas distribution may be used as a critical control point. In that case an upper time interval could be 5 min. The reason ventilation should be turned off prior to gas distribution is to minimise carbon dioxide leakage and ensure that a sufficient concentration is reached. A technical argument in favour of turning the ventilation off in advance, is that an increased temperature will result in faster evaporation of CO_2 and limit the temperature drop. However, the standard temperature of a poultry barn is normally high enough to achieve rapid CO_2 evaporation without any extra increase in temperature. Furthermore, a rapid increase in temperature may be as stressful to the hens as a rapid decrease and is also often accompanied by a rapid increase in humidity and a deterioration of air quality, further increasing stress for the birds. Hence we discourage such a practice.

Some veterinarians do not understand that 'fencing off the gas inlet' is for the protection of the birds. They note that all staff are standing back (which is certainly also important but not from the perspective of animal welfare). From the results we see a substantial need for information to both farmers and veterinarians to ensure that euthanasia is carried out in accordance with the legislation and recommended routines.

In many poultry barns direct inspections are not possible since houses are not provided with windows. The veterinary reports showed that the time taken before the veterinarians checked whether the hens were dead or not ranged from 20 min to more than 1 h. However, in most instances, this information is not available from the protocols, and a decision should be made as to whether or not the veterinarian should be able to leave the farm before ensuring and confirming that the birds are actually dead. The possibilities of using simple web cameras instead of windows should be investigated.

The protocol asks whether the hens are in an acceptable condition from an animal welfare perspective. In none of the reports was there any note about the birds' condition. We suggest that the reason for this data needs explanation or, if irrelevant, the question should be deleted. Veterinarians may also need guidance on evaluating the welfare of laying hens at flock level.

In this protocol there is no overall question as to whether or not the killing was carried out in an humane way. As there is ample opportunity for making mistakes this seems a relevant question to include in a protocol for controlling animal welfare during CO_2 killings.

Animal welfare implications and conclusion

Killing of birds, regardless of the method chosen, does involve some degree of stress or suffering. No birds in this study were killed or handled for the purpose of the study as all killings would have been carried out in any case. The protocol used by the inspecting veterinarians is designed to allow for an immediate evaluation of bird welfare by the Central Competent Authorities, who may choose to take relevant action in case of non-compliance. For this study these protocols were analysed 2–4 years after the killings were performed.

- The bird environment becomes hostile during CO_2 killings. When trucks carrying carbon dioxide unload the gas into the barn through smaller-sized gas inlets a forceful jet of very cold gas appears, creating a fog inside of the barn. The force of the gas jet however, decreases fairly rapidly some distance away from the inlet, where a more gradual increase in CO_2 concentration and decrease in temperature appears. We found no signs of temperature-related lesions.
- The birds first react with avoidance, ie moving away from the gas inlet. Thereafter, there is a brief period of relative calmness, before the respiration of the birds is affected. Gasping, vocalisation and convulsions have been proposed

as bird welfare indicators and their suitability is confirmed by this study. Determining where the ethically acceptable levels and times for such behaviours should be set is, however, beyond the scope of this study.

• In a majority of the cases the carbon dioxide, whole-house gas killing of spent hens in Sweden was carried out in compliance with the legislation. According to the reports the birds became unconscious within 10–20 min from the start of gas distribution and death of the birds was achieved reliably.

• In roughly one-quarter of the reported cases, killings were not performed in total compliance with the legislation, mainly in relation to shortcomings in fulfilling formal requirements. Furthermore, there appears to be some systematic under-reporting to the Swedish Board of Agriculture.

• There are cases where non-compliance and mistakes have led to actual or potential threats to bird welfare, and further information and training of egg producers and veterinarians may be necessary to avoid similar incidents in the future.

Suggestions for improvements to the present method, apart from improving compliance with already existing guidelines:

• The recommendation of adding multi-point gas injection sites, which may benefit an even gas distribution and minimise the drop in temperature, should be emphasised.

• If this method is to be routinely used, a device for measuring the carbon dioxide (or oxygen) concentration in the barns should be a requirement. This is to avoid unnecessary risks for people working with depopulating the barn after the killings and also for veterinarians inspecting the euthanasia. Furthermore, in-house temperature should be monitored continuously.

• As this method of euthanasia has several potential animal welfare hazards we conclude that the presence of a veterinarian, or another competent observer, is a well-founded requirement which should remain in the legislation.

• The design of the reporting forms and the routines surrounding the veterinary reporting should be clarified and the purposes of recording the different parameters should be stated.

• Last, but not least, the farmers have the greatest responsibility for the welfare of their birds. To minimise any risks to animal welfare when killing spent laying hens in the barn using carbon dioxide, all aspects of the legislation must be complied with, as must instructions from the gas delivery company.

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