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INDIVIDUAL AND GROUP EUTHANASIA IN FARMED MINK*

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Abstract

The study seeks to evaluate the effectiveness of euthanasia in mink (*Neovison vison*). The gases studied were filtered exhaust CO, and cylinder CO and CO_2. The experimental setups were as follows: (1) individual killing of mink in a small killing box (35 × 30 × 60 cm; H × W × L); (2) individual killing of mink in a small killing box, kept inside a killer (12.5 × 12.5 × 55 cm; H × W × L); (3) group killing of mink in a large killing box (75 × 75 × 150 cm; H × W × L); and (4) group killing of mink in a large killing box (70 × 70 × 125 cm; H × W × L). The results showed that filtered exhaust CO concentrations of 1.2–3% were too low. Concentrations of 4–6% were effective. With cylinder CO of 4% the killing time was the same as that with engine CO at 4%. A cylinder CO_2 concentration of ≥ 80% was effective. The shortest time interval after the end of all movements and respiration was with cylinder CO_2. No difference was found between cylinder and filtered exhaust CO. The time the animals were alive did not significantly differ between the groups ($P>0.05$). The first animal collapsed soonest among those inhaling CO_2 (CO_2 vs cylinder CO; $P=0.0045$; and CO_2 vs filtered exhaust CO; $P=0.0009$). No difference was found between cylinder CO and filtered exhaust CO ($P=0.4654$). There was also a significant difference in the time it took for the last animal to collapse. Here again the CO_2 animals collapsed the soonest (CO_2 vs cylinder CO, $P=0.0062$; CO_2 vs exhaust CO, $P=0.0012$). No difference was found between cylinder and filtered exhaust CO ($P=0.5660$). It is concluded that concentrations of CO ≥4% and of CO_2 ≥80% in the euthanasia box are sufficient to kill effectively.

Key words: *Neovison vison*, killing, euthanasia, animal welfare, fur farming

Several methods of euthanasia have been used over the years to kill farmed mink (*Neovison vison*). These include neck breaking, electric shock, inhaling carbon monoxide (CO), carbon dioxide (CO_2), nitrogen (N_2), argon (Ar), and intravenous lethal injections (Loftsgård et al., 1972; Loftsgård, 1980; Finley, 1980; Gierløff, 1980; Löliger, 1984; Lamboy et al., 1985; Hansen et al.,1991; Cooper et al., 1998; Raj

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and Mason, 1999; EFBA, 1999). The main technique currently used is the inhalation of gas, most commonly either CO_2 or CO. Both are commercially available in compressed form in a cylinder. CO can be also produced by a petrol-driven engine. This technique has been favoured in several European countries (Korhonen et al., 2011 a).

Even when the euthanasia method used is the best possible currently known, the procedure can still cause pain, distress, anxiety or apprehension. Every effort must therefore be made to ensure that the animals are killed as quickly and painlessly as possible (Council of the European Union, 2009; EFBA, 2009). The criteria for a painless death can be established only after the mechanisms of pain are understood. Pain can be assumed to be the sensation that results from nerve impulses reaching the cerebral cortex via ascending neural pathways (AVMA, 2007; Korhonen et al., 2011 b). For pain to be experienced, the cerebral cortex and subcortical structures must be functional. If the cerebral cortex is nonfunctional, pain is not experienced. Knowledge of the functional state of the brain is therefore essential for animal well-being during euthanasia.

We currently lack conclusive information on mink euthanasia with either pure source CO from a cylinder or filtered exhaust CO from a petrol-driven engine. Further information is also needed on euthanasia with CO_2. The present study is part of a research project evaluating the humaneness of gas euthanasia in mink (Korhonen, 2010). The project includes a questionnaire study examining the current use of euthanasia gases in farming practice (Korhonen et al., 2011 a), and an electrophysiological study on CO and CO_2 euthanasia (Korhonen et al., 2011 b). In the latter study, electrocardiography (ECG), electroencephalography (EEG), brainstem auditory evoked responses (BAER) and respiratory rate were clarified before and during euthanasia. Animals had to be sedated to measure electrophysiological indices in detail, and so the behaviour of the animals could not be observed. However, behavioural data are valuable when making evaluations of the dying process (Chalifoux and Dallaire, 1983; Coenen et al., 1995). Behavioural manifestations are particularly important for farmers who have to make decisions concerning the death of their mink in practice. They have to understand the criteria for confirming death. In the present study, mink were not sedated but were euthanized while in a normal living body condition. We were thus able to record their behavioural reactions during the dying process.

Material and methods

Four different experimental setups were used: (1) individual killing of mink in a small killing box (35 × 30 × 60 cm; H × W × L) without a killer; (2) individual killing of mink in a small killing box, kept inside a killer (12.5 × 12.5 × 55 cm (H × W × L); (3) group killing of mink in a large killing box (75 × 75 × 150 cm; H × W × × L) without a killer; and (4) group killing of mink in a large killing box (70 × 70 × × 125; H × W × L) without killer. The smaller killing box had one transparent glass pane on the front wall and one on the roof. Larger killing boxes had two transparent glass panes on two sides and one pane on the roof. The use of transparent glass made visual inspection and video recording of animals possible. Experiments 1–3 were performed at MTT Kannus, Finland during March and November 2010. Experiment 4 was made at Edelveen research farm, The Netherlands during 28–29 of March, 2011.The euthanasia gases studied in Exps. 1–3 were (1) filtered exhaust CO; (2) cylinder CO; and (3) cylinder CO_2; and in Exp. 4 cylinder CO. Exhaust gas CO was produced by a feeding truck (Minkomatic 14 HP) in each experiment. The larger killing box had an automatic closing device on the hatch through which the animals enter the box.

In Exps. 1–3, the CO concentration in the box was measured with a BINOS 1 CO and CO_2 analyser (Leybold-Heraeus, Germany). CO_2 was measured with a DAT-2 Special Tiedonkeruu-datalogger (Pietiko Oy, Turku). CO concentration in Exp. 4 was measured by Flue Gas Analyser (Testo 350 XL, Germany). Concentrations in the laboratory air were measured by KANE 100-1 CO/CO_2- analyser (UK). Engine-produced CO was filtered with a Sundström P3R5X 5R 510 filter (Exps. 1–3). In Exp. 1, behavioural observations of the dying process were made visually. In Exps. 2–4, behaviour was recorded with a Canon Digital Video Camera MV 900. A microphone was placed inside the box (Exps. 3–4) to record any sounds (Olympus VN-6800PC Digital Voice Recorder China). Analyses of the gas composition in the feeding machine, killing trolley and car were made at a local garage (Niemi-Korpi Oy, Kannus) according to the motor-vehicle exhaust inspection test. The analyses cover four different components of the exhaust gas, i.e., carbon monoxide (CO), carbon hydrate (CH), carbon dioxide (CO_2) and oxygen (O_2).

In Exps. 1 and 2, the animals were placed in the killing box before gas delivery was started. In Exp. 3, the appropriate gas concentration was introduced into the box before the animals were placed inside it. In Exp. 4, gas concentration in the killing box was around 2–3% before the first animal was placed inside. Body weights were measured on a Vaakakoskinen AD-4326A balance. Heart function was assessed by palpation when gassed animals were taken out of the killing box. Statistical analyses were conducted with the SAS system for Windows 9.1. Pair-wise comparisons were made with Tukey's test.

Results

Table 1 shows how different gas concentrations affect the dying process. The data are based on behavioural recordings of the animals and should therefore only be considered as estimates. With filtered exhaust CO, concentrations of 1.2–3% were too low, as the mink either did not die at all or took a long time (>7–15 min) to do so. Concentrations of 4–6% seem to be effective, causing death of the animal within 3–6 min of the start of gas input. With cylinder CO, only a 4% concentration was tested. The killing time was then about the same as that with filtered exhaust CO at 4%.

Table 1. Effects of gas concentration on dying in mink. Data are based on visual observations. N=number of animals. Size of killing box: 35 × 30 × 60 cm; H × W × L

N	Body weight (g)	Concentration (%)	Max T (°C)	Restless (min)	Hyperventilation (min)	Death (min)
Exhaust CO:						
1	2403	1.2	10.4	4.5	12	>15
2	2205–2810	2	9.2–10.1	2.5–3.5	4–9	>7–10
4	1778–2617	3	6.8–8.4	2.5–3	4–9	>7–10
3	1541– 2319	4	7.6–10.1	2.5	2–5	3-4
1	2103	5.5	9.0	2.5	3–4	≤5
1	2428	6	7.4	1	1–2	≥3
Cylinder CO:						
8	1386–2223	≥4	12.2–16.5	1–2	4–5	4-6
Cylinder CO_2:						
1	1200	70	-	≥1	≤2	>
1	1150	75–80	-	<1	<2	5.5
8	1120-–430	80	12.6–14.8	<1	1–2	4–5
2	965–1285	>85	-	1	1.5	≥3

A cylinder CO_2 concentration of 70% was not able to kill the mink within 7 min. A concentration of ≥80% was effective, the average time to death being 4–5 min after the start of gas input. Typical behaviours before death were restlessness, hyperventilation, uncoordinated movements and recumbency. Screaming or other disturbed vocalization was slight. Sneezing or coughing was not found during exposure.

Table 2 shows the behavioural reaction of non-sedated mink to the gases studied. Behavioural recording began after the start of gas input into chamber. However, it was not very easy to determine through observation exactly when each behavioural process began or finished. Thus these data are mere estimates and provide only a rough framework for behaviour intervals during gas input.

The shortest time interval after movement and respiration ceased was found for cylinder CO_2. No difference was found between cylinder CO and filtered exhaust CO. The result was the same for hyperventilation. When hyperventilating, the animals first breathe deeply and rather quickly, then less rapidly and finally cease breathing altogether. Screaming or other disturbed vocalization was rare. Sneezing or coughing was not observed during exposure to gases.

Table 3 gives the results of group killing experiments. Animals were not placed in the killing box until an appropriate gas concentration had been achieved.

The time the euthanasia group remained alive did not significantly differ between the experimental groups ($P>0.05$). The same held true for the killing time of the last animal (Table 3). However, the first animal fell/lay down soonest in the CO_2 group (CO_2 vs cylinder CO; $P=0.0045$; and CO_2 vs filtered exhaust CO; $P=0.0009$). No difference was found between cylinder CO and filtered exhaust CO ($P=0.4654$).

There was also a significant difference in the time it took for the last animal to fall/ lie down. Here again the CO_2 animals collapsed the soonest (CO_2 vs cylinder CO, P=0.0062; CO_2 vs filtered exhaust CO, P=0.0012). No difference was found between cylinder CO and filtered exhaust CO (P=0.5660). In general, mink appeared to be surprisingly calm and non-aversive in the killing box. Screaming or other disturbed vocalization was rare. Sneezing or coughing was not found during exposure inside the killing box.

Table 2. Individual phases of euthanasia in non-sedated male mink. Time in seconds. The animal was placed inside a killer in a killing box. Size of killer: 12.5 × 12.5 × 55 cm (H × W × L). Size of killing box: 35 × 30 × 60 cm; H × W × L. Estimated from behavioural patterns recorded under laboratory conditions. Exhaust CO and Cylinder CO concentrations 4%, cylinder CO_2 concentration 80%

Behaviour	Exhaust CO	Cyl CO	Cyl CO_2
Active/restless	0–69	0–75	0–57
Hyperventilation	69–240	75–249	57–186
All movements and respiration ceased	240–303	249–306	186–192
Number of animal	5	6	5

Table 3. Data on group euthanasia of non-sedated mink at pelting. Size of killing box: 75 × 75 × 150 cm; H × W × L. N=4 video recording takes for each gas setup studied. Time is as seconds (mean ± SE). Exhaust CO and cylinder CO concentrations 4%, CO_2 concentration 80%

	TAKE 1	TAKE 2	TAKE 3	TAKE 4	MEAN
Exhaust CO:					
males	14	11	18	17	15±1.6
females	16	23	17	15	17±1.8
total size of group	30	34	35	32	33±1.4
time group alive (sec)	355	303	433	300	347±31
1st animal falls/lies down (sec)	59	69	71	99	75±8.6
last animal falls/lies down (sec)	128	70	67	84	87±14
killing time of last animal (sec)	268	244	233	179	231±18
Cylinder CO:					
males	18	17	20	15	18±1.0
females	17	16	15	14	16±0.7
total size of group	35	33	35	29	33±1.4
time group alive (sec)	432	329	306	309	344±29
1st animal falls/lies down (sec)	83	53	64	50	63±7.4
last animal falls/lies down (sec)	101	67	65	57	73±9.7
killing time of last animal (sec)	241	202	195	217	214±10
Cylinder CO_2:					
males	17	17	16	17	17±0.3
females	18	16	17	16	17±0.9
total size of group	35	32	32	32	33±0.8
time group alive (sec)	262	253	326	364	301±26
1st animal falls/lies down (sec)	14	22	29	14	20±3.6
last animal falls/lies down (sec)	14	14	18	10	14±1.6
killing time of last animal (sec)	133	139	202	243	179±26

In Exp. 4, body weights of animals averaged 2.58±0.31 kg. The aim of the trial 1 was to perform the killing procedure according to the present Dutch farming practice. Altogether 36 male mink were euthanized. Gas flow was initially 2.0 l/min for 2 first minutes. After 2 min the CO concentration in the box was 2.5% (Fig. 1). Thereafter, first male mink was placed into chamber. Gas flow was running through the experiment (at rate of 1.0 l/min). After 6.5 min and 7.5 min the CO concentrations increased to 4.3% and 4.9%, respectively. Last male mink was placed into the box 8 min after the start. Concentration remained after that ≥5%. Evaluation of behaviour during gas exposure showed that animals were calm. No signs of agitation were observed. After 2 min since last animal was inside (13.6 min), the gas flow was closed and killing box was opened. All animals were found dead.

In trial 2, the gas flow was initially 2.0 l/min for 2 first minutes. After 2 minutes the CO concentration was 1.7%. Altogether 39 males were put one by one into the killing box. The CO concentration was kept between 2.5 and 4% during the killing process (Fig. 1). Last animal was dropped into the box since 9.6 min of the start. After 15.1 min the box was emptied. One mink was found to be alive yet.

Trial 3 included 44 male mink. Animals were placed into the box one by one after initial flow of 2.0 l/min for 2 minutes. CO concentration was 2% after 4 min (Fig. 1). Concentration was kept between 2 and 3% during the rest of trial 3. After 22.3 min of the start the box was emptied. Five animals were found to be alive yet.

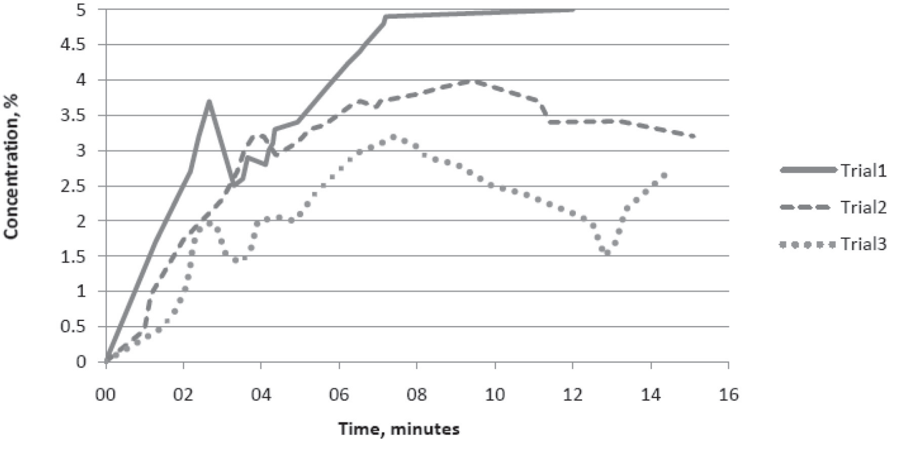

Figure 1. Development of CO concentration (%) in the killing box in Exp. 4

Discussion

The most accurate way to evaluate animals' dying process and the moment of death is to perform a detailed electrophysiological analysis under laboratory conditions. This was done in our previous study (Korhonen et al., 2011 b), which summarized the results of electrophysiological indices on euthanasia of farmed mink with CO and CO_2 gases. The death of animals in that experiment was confirmed by accurate measurements of electroencephalography (EEG), electrocardiography (ECG), brainstem auditory evoked responses (BAER) and respiratory rate. The results showed that the gases studied first affected the brain and brainstem, which was seen as loss of EEG and BAER and, thereafter, respiration and heart rate in turn. Painful effects can only be experienced when the brain function of the animal is intact (Jepsen et al., 1981; Hansen et al., 1991; AVMA, 2007). EEG recordings showed a clear decline in the cerebral cortex during gas exposure. In farming practice, however, the death of mink cannot be confirmed by accurate scientific electrophysiological measurements; farmers have to rely on behavioural observations and determine the state of death by their subjective criteria. However, behavioural analyses of dying and the moment of death are not so exclusive as are electrophysiological ones (cf. Lamboy et al., 1985; Hansen et al., 1991).

In the present study, the euthanizing agent was a gas, either CO or CO_2. The main principle for proper killing here is that any gas that is inhaled must reach a certain concentration in the alveoli before it can be effective. Therefore, euthanasia with gases always takes some time and often lasts longer than certain other euthanasia methods. The humaneness of a particular inhalant gas depends on whether an animal experiences distress between the time it begins to inhale the agent and the time it loses consciousness (AVMA, 2007).

Exp. 1 clearly showed that the concentration of the gas studied markedly affected the effectiveness of the killing process. According to the EU regulation to be implemented in 2013 (Council of the European Union, 2009) the concentration of filtered exhaust CO for euthanasia must exceed 1%. Our results showed that concentrations of ≤3% are too low to kill mink properly. Similarly, Carding (1977) reported that a CO concentration of 3% is not effective to kill dogs, whereas a concentration of 4–6% is effective. Likewise, AVMA (2007) reported that death by CO occurs rapidly at concentrations of 4–6%. In the present study, filtered exhaust CO at 4% or more seemed to work well for euthanasia of mink, too. The recommended cylinder CO concentration in the EU regulation is no less than 4%. This is an effective level and seems to kill mink within about the same time as does filtered exhaust CO at 4%. A CO_2 concentration of 70% was not enough to kill mink properly. This coincides well with the finding of Hansen et al. (1991) that CO_2 at 70% was insufficient. The recommended cylinder CO_2 concentration in the EU regulation is 80%. According to our present findings, this concentration is effective for mink euthanasia.

Exp. 2 in the present study was performed under the same laboratory conditions as was our electrophysiological study (Korhonen et al., 2011 b), so we can compare the physiological and behavioural variables to a certain extent. The studies differed in that the animals were sedated in the electrophysiological study but not in Exp. 2. Our behavioural observations of non-sedated mink are rough estimates of behaviour during the dying process but they seem to coincide with our electrophysiological indices fairly well. This is an important finding and suggests that the results for sedated and non-sedated animals are largely comparable.

Exp. 3 showed that both filtered exhaust CO and cylinder CO are equally effective when killing mink in groups. In terms of the total killing time of a group, all three gas methods studied were effective. However, other variables tempt us to conclude that the most appropriate method is cylinder CO_2. In particular, the time required for the first and last animal to fall/lie down (collapse) was very short with CO_2. Loss of movement coordination and a natural standing position (collapse) is a clear sign of loss of consciousness (Blackshaw et al., 1988; Hansen et al., 1991; AVMA, 2007). The short time for an animal to collapse by using CO_2 tempts us to conclude that mink lose consciousness more rapidly with CO_2 than with CO. This conclusion is supported by our previous electrophysiological measurements (Korhonen et al., 2011 b), which revealed that disappearance of EEG was most rapid with cylinder CO_2. Likewise, Hansen et al. (1991) reported that movements became uncoordinated more rapidly in animals killed with CO_2 than in those killed with CO. Rapid collapse suggests also a sedative effect, diminishing sensitivity to pain (Blackshaw et al., 1988).

Exp. 4 revealed that the way the Dutch farmers in practice are killing mink by using cylinder CO is effective. Although the CO concentration at the time when first animal was dropped into chamber was still less than 4%, the concentration at regular flow led to continuously increased concentration. At the latter part of killing procedure the CO already was ≥5%. This finally resulted in the proper death of animals. Exp. 4 also confirmed the previous findings of our electrophysiological study (Korhonen et al., 2011 b) that CO concentration of 2% is too low to kill mink properly. Results of Exp. 4 also agreed well with the results of Exp. 1 showing that concentration lower than 3–4% is not effective enough for killing.

During the dying process mink appeared surprisingly calm and did not show any actual signs of panic, fear or excitation. Clearly the animals do not sense the gases before their brain activity is already at least partly diminished. This is typically a state of non-pain (Korhonen et al., 2011 b). Hence, they do not experience the killing situation as stressful or otherwise as a cause of agitation or distress.

According to Cooper et al. (1998) exposure of mink to CO_2 may cause sneezing and coughing. They used passive avoidance technique and concluded that mink find CO_2 aversive. In the present study, no signs of sneezing or coughing of mink were seen during killing procedure. According to our experiments, CO_2 as well as CO, cannot be considered highly aversive to mink.

The animals' behaviour during gas exposure suggests that euthanasia gases do not contain any marked irritating components. Irritant impurities in engine-produced CO typically account for less than 1% of the total concentration (Laurikko, 1993). Furthermore, we used a filter for exhaust CO to further diminish the concentration of irritating components. Lamboy et al. (1985) pointed out that use of a proper filter results in a relatively non-irritating CO gas. Also Carding (1977) considered a filter important for removing irritant particles. As for pure source CO, irritant impurities are quite likely almost zero. The concentration of impurities in pure CO_2 is also very low and so cannot cause appreciable irritation.

The killing effect of exhaust gas is unlikely to be due to CO alone as the gas also contains CO_2, O_2 and HC. According to Von Oettingen (1944), the toxicity of CO is exacerbated by an increase in CO_2. As our findings showed, engine-produced CO always also contains CO_2 (2.6–8%), which may increase the effectiveness of the killing process.

Furthermore, inhalation of CO_2 at a concentration of ≥7.5% raises the pain threshold, and higher concentrations of CO_2 have a rapid anesthetic effect (Leake and Waters, 1929; Woodbury et al., 1958; AVMA, 2007). Moreover, Coenen et al. (1995) have reported that, in rats, signs of agitation and asphyxia were almost completely absent when oxygen was added to the CO_2 exposure.

In conclusion, the concentration of the gas in the killing box is a critical factor in the humaneness of euthanasia. Our present results suggest that concentrations of CO ≥4% and of CO_2 ≥80% in the euthanasia box are sufficient to kill mink effectively. Filtered exhaust CO and also CO and CO_2 compressed in cylinders can be used.

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Indywidualne i grupowe uśmiercanie norek z chowu fermowego

STRESZCZENIE

Celem badań była ocena skuteczności eutanazji u norek (*Neovison vison*). Badanymi gazami były przefiltrowane spaliny CO, oraz CO i CO_2 z butli. Zastosowano następujące układy doświadczalne: (1) uśmiercanie pojedynczych norek w niewielkim boksie do eutanazji (35 × 30 × 60 cm; wys. × szer. × × dług.); (2) uśmiercanie pojedynczych norek w niewielkim boksie do eutanazji, umieszczonych wewnątrz urządzenia do uśmiercania (12,5 × 12,5 × 55 cm; wys. × szer. × dług.); (3) grupowe uśmiercanie norek w dużym boksie do eutanazji (75 × 75 × 150 cm; wys. × szer. × dług.); (4) grupowe uśmiercanie norek w dużym boksie do eutanazji (70 × 70 × 125 cm; wys. × szer. × dług.). Stwierdzono, że stężenie przefiltrowanych spalin CO wynoszące 1.2–3% było zbyt niskie, natomiast stężenie 4–6% było skuteczne. Przy stężeniu CO z butli wynoszącym 4% czas uśmiercania był taki sam jak przy stężeniu CO z silnika wynoszącym 4%. Koncentracja CO_2 z butli wynosząca ≥80% była skuteczna. Wszelkie ruchy i oddychanie zwierząt ustawały najszybciej przy zastosowaniu CO_2 z butli. Nie stwierdzono różnicy pomiędzy CO z butli a przefiltrowanymi spalinami CO. Nie było istotnych różnic w czasie przeżycia zwierząt pomiędzy grupami ($P>0,05$). Zwierzęta najszybciej traciły przytomność wskutek wdychania CO_2 (CO_2 vs CO z butli; P=0,0045; CO_2 vs przefiltrowane spaliny CO; P=0,0009). Nie stwierdzono różnicy pomiędzy CO z butli a przefiltrowanymi spalinami CO (P=0,4654). Zaobserwowano również istotną różnicę w czasie, w którym ostatnie zwierzę traciło przytomność. Także w tym przypadku zwierzęta poddane działaniu CO_2 najszybciej traciły przytomność (CO_2 vs CO z butli, P=0,0062; CO_2 vs spaliny CO, P=0,0012). Nie stwierdzono różnicy pomiędzy CO z butli a przefiltrowanymi spalinami CO (P=0,5660). W konkluzji stwierdza się, że stężenia CO wynoszące ≥4% oraz stężenia CO_2 wynoszące ≥80% zastosowane w boksie do eutanazji są wystarczające do skutecznego uśmiercania.