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ELECTROPHYSIOLOGICAL STUDY ON CO AND CO_2 EUTHANASIA IN MINK (*MUSTELA VISON*)

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Abstract

The aim of the present study was to define at what time point animal is dying and how quickly changes in the function of brain and heart can be observed. Four groups of sedated standard dark male mink (*Mustela vison*) were tested: euthanasia with filtered exhaust gases (CO concentration in the killing box 4%, 8 animals), carbon dioxide (CO_2 from a cylinder, concentration in the killing box 80%, 8 animals), carbon monoxide (CO from a cylinder, concentration in the killing box 4%, 9 animals) and euthanasia with carbon monoxide (CO from a cylinder, concentration in the killing box 2%, 6 animals). Brainstem auditory evoked responses (BAER), electroencephalography (EEG), electrocardiography (ECG) and respiratory rate were measured before and during euthanasia. Mean time of decline/absent BAER was 112/176, 138/183, 235/390 and 528/833 seconds after gas application in groups, respectively. Mean time for first changes/absence of EEG was 42/86, 39/75, 55/190 and 176/426 seconds after gas application in groups, respectively. Mean time for first changes in respiration/absent breathing was 42/217, 28/227, 144/477 and 331/901 seconds after gas application in groups, respectively. Mean time for first changes/absence of ECG was 105/292, 117/220, 215/289 and 481/682 seconds after gas application in groups, respectively. Our results indicate that the studied gases first affect brain and brainstem which was seen as loss of EEG and BAER and just thereafter respiration and heart rate in turn. While sensitivity to pain is essentially related to consciousness and function of brain, gases can be considered to primarily and effectively lead to state of non-pain. Particularly the death with the filtered exhaust CO and the cylinder CO_2 gases occurs quickly and in very comparable times. The euthanasia with the cylinder CO of 2% concentration seems to be too long and is most likely not suitable for the mink euthanasia in general. Observable signs of marked irritation or aversion were not found during exposure to studied CO and CO_2 gases.

Key words: gas exposure, killing practice, animal welfare, fur farming, culling

Several methods and techniques have been considered for the proper euthanasia of farmed mink (*Mustela vison*). These include neck breaking, euthanasia with electricity, carbon monoxide (CO), carbon dioxide (CO_2), nitrogen (N_2), argon (Ar) and various lethal injections (Loftsgård et al., 1972; Loftsgård, 1980; Finley, 1980; Gi-

erløff, 1980 a, b; Löliger, 1984; Lamboy et al., 1985; Hansen et al., 1991; Cooper et al., 1998; Raj and Mason, 1999; EFBA, 1999; Fitzhugh et al., 2008). Among those, neck breaking is today prohibited. Euthanasia with electricity has been employed to a certain extent, but it is not a favoured method for today's practice and, therefore, not actually used. It would also require some alternative method for an additional assurance of death. Exposure to gases is favoured in the mink. The predominant killing gas on European fur farms is carbon monoxide (CO), which originates either from a petrol machine (filtered exhaust gases) or, alternatively, from a cylinder (pure source). However, the use of cylinder carbon monoxide is still rather infrequent. According to the statistics of the European Fur Breeders' Association (EFBA, 2008), it is mainly used in the Netherlands, Germany, Greece and France. The other common cylinder gas employed for killing the mink is carbon dioxide (CO_2) (Korhonen et al., 2011 a). Its use seems to be increasing.

At present, there is not enough accurate information available on humane mink euthanasia with pure source (cylinder) and filtered exhaust carbon monoxide (coming from a petrol engine) and cylinder carbon dioxide, on the method, on the functionality of the method, and on trackable variables. Therefore, more focused scientific research and background information on relevant gas euthanasia of farm-raised mink is definitely needed.

The euthanasia of farm mink (*Mustela vison*) with filtered exhaust gases (the engine induced CO) and with carbon monoxide and carbon dioxide from a cylinder (the bottle administered CO and CO_2) were compared in this study. The aim was to define at what time point animal is dying and how quickly changes in the function of brain and heart can be observed. Essential here is to understand the onset of unconsciousness while painful sensations can be only experienced when the brain function of the animal is intact. Pain can be assumed to be the sensation that results from nerve impulses reaching the cerebral cortex via ascending neural pathways (cf. Jepsen et al., 1981; Hansen et al., 1991; AVMA, 2007). For pain to be experienced, the cerebral cortex and subcortical structures must be functional. Therefore, the functional state of brain is essential for animal wellbeing during euthanasia.

Material and methods

Experimental groups

The experiment was performed at MTT Kannus, Finland in mid-April 2010. Four groups of male standard dark mink (*Mustela vison*) were tested in this study: (1) euthanasia with filtered exhaust gases (combination of CO, CO_2, HC, O_2); CO concentration in the killing box 4%, N=8 animals; (2) euthanasia with carbon monoxide (CO) from a cylinder, concentration in the killing box 4%, N=9 animals; (3) euthanasia with carbon monoxide (CO) from a cylinder, concentration in the killing box 2%, N=6 animals; (4) euthanasia with carbon dioxide (CO_2) from a cylinder, concentration in the killing box 80%, N=8 animals. Body weights of animals were measured before the start of experiment (Vaakakoskinen AD- 4326A balance). They

were fed and kept according to normal farming procedures. The health of animals was visually checked daily before study.

Pre-euthanasia treatments

Animals were sedated with an i.m. injection (Jepsen et al., 1981). The otoscopy was performed in order to evaluate the external ear canal and the tympanic membrane. The animal's heart rate and breathing as well as palpebral, corneal and withdrawal reflexes were examined before placing the animal into the wooden-glass chamber (60 × 30 × 35 cm; L × W × H), and once before euthanasia with CO and CO_2.

Animals were placed into the wooden-glass chamber with the openings for the wires of subcutaneous needle electrodes for recording of brainstem auditory evoked potentials (BAER), electroencephalography (EEG) and electrocardiography (ECG). Brainstem auditory evoked potentials were recorded on every side twice before euthanasia. Simultaneously EEG and ECG recordings of 5 minute duration were performed before euthanasia with CO and CO_2 in order to record the live EEG pattern.

At and post-euthanasia treatments

The mink were euthanized in a wooden-glass chamber measuring 60 × 30 × 35 cm (L × W × H), which makes inspection and video recording possible. The course of euthanasia was recorded with a video camera (Canon MV900). CO and CO_2 concentrations in the chamber were measured by using Leybold-Herauens Werk Hanau (Germany) analyser. CO and CO_2 concentrations in the ambient air were measured by using KANE 100-1 CO/CO_2-analyser (UK). Gas concentration, temperature and gassing time were evaluated during the experiment.

The animals were immobilized in the neck and the tail area and gas euthanasia was performed while continuously recording BAER, EEG and ECG. BAER and EEG recordings were continued until the brain death/no brain activity was recognized. ECG was performed until the heart arrest (asystole) was detected. The animal's heart rate, breathing, palperal, corneal and withdrawal reflexes were examined after discontinuation of the BAER, EEG and ECG recordings and after removal of the animal from the chamber.

Results

Pre-euthanasia treatments

Healthy male mink in good condition were included in the study. Body weights of animals varied between 1307 and 2298 g. The animals were sedated with the combination of 0.4 mg medetomidine (Dorbene 0.4 ml, 400 mcg) and 10 mg tiletamine with 10 mg zolazepam (Zoletil 0.2 ml). The combination of Dorbene (0.4 ml) and Zoletil (0.2 ml) was mixed within one syringe and injected intramuscularly in all animals.

Otoscopy evaluated in all studied animals revealed the external ear canals and the tympanic membranes to be normal. The heart rate ranged between 90–294 beats

per minute (mean 194) and breathing frequency was 16–72 breaths per minute (mean 40). Palpebral, corneal and withdrawal reflexes were absent in all 31 animals before they were placed into the wooden-glass euthanasia chamber. All animals were placed into the euthanasia chamber and subcutaneous needle electrodes for the recording of BAER, EEG and ECG were inserted. Brainstem auditory evoked potentials were recorded simultaneously with EEG and ECG recordings before euthanasia for about 5 minutes.

BAER was performed after placing the earplug-loudspeakers deep into the external ear canal. The alternating click stimuli of 90 decibels sound pressure level (dB SPL) were delivered at a frequency of 10 Hz. A masking noise of 50 dB SPL was applied to the contralateral ear. A thousand clicks were averaged for each ear. In all 31 animals BAER was recorded successfully before euthanasia as illustrated in the image (Figure 1).

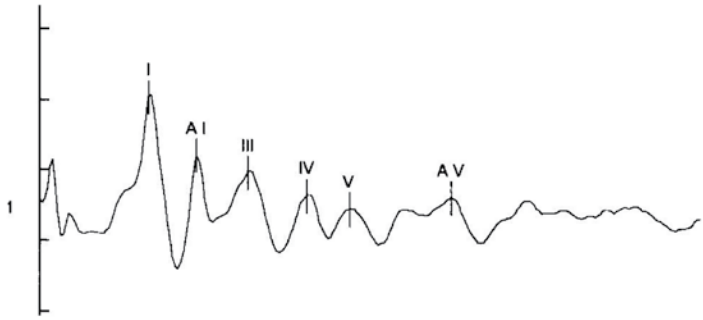

Figure 1. The normal BAER recording of the right ear before euthanasia. Six recognizable peaks can be identified

The side of the post-euthanasia BAER recording was chosen based on the pre-euthanasia recording: the ear with less disturbance, clearly defined peaks and larger amplitude were chosen for the later recording. If both ears were similar the right ear was recorded during euthanasia. From the 31 animals 5 times the left and 26 times the right ear was recorded during gas euthanasia. Portable EBNeuro equipment for the EEG recordings was used. EEG was performed in all animals simultaneously with the BAER recording.

Animals were positioned in a sternal recumbency in euthanasia chamber, no additional fixation in the neck or tail area was needed as animals were sleeping without motion. Standardized placement of EEG electrodes resembling the 10–20 international system for humans was used. A 2-channel reference montage (C3, C4; sensitivity = 5 μV/mm; time constant = 0.3 sec; Hf = 70 Hz; notch filter inserted; ground: caudally to the external occipital protuberance) was used to record EEG. Three EEG needles were inserted as subdermal active, and ground electrodes. Impedances did not exceed 5 kΩ. EEG recording lasted 4–5 minutes before gas application and recording was continued till brain activity could not be recognized any more (isoelectric EEG lines). EEG data were stored in the acquisition station for subsequent visual analysis.

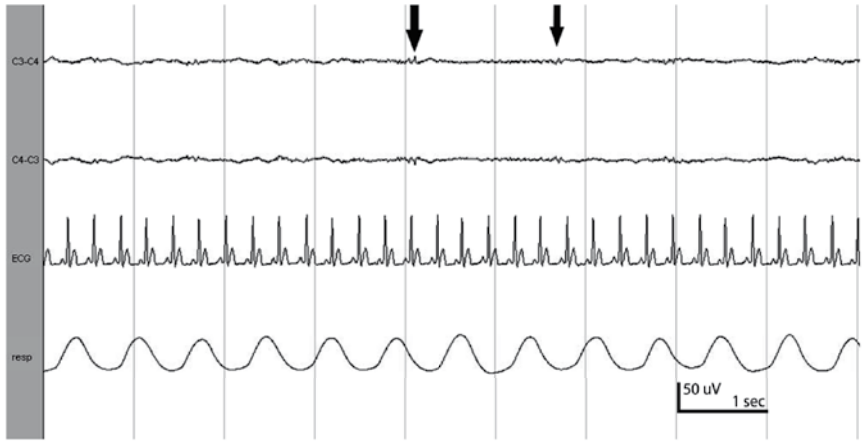

Figure 2. Normal EEG (C3-C4; C4-C3), ECG and breathing pattern (resp) in sedated mink before euthanasia. Slow background activity (delta and theta) is superimposed with sleep spindles (arrow). Bar: sensitivity 50 uV/cm, 1 sec 3 cm

The ECG and respiratory rate (RR) were recorded via the polygraphic electrodes of the EEG equipment (ECG: sensitivity = 70 μV/mm, time constant = 0.3 sec, Hf = 70 Hz; RR: sensitivity 20–50 μV/mm, time constant 0.3 sec, Hf = 70 Hz). The polygraphic electrodes were connected to volumetric transducer, which was applied around the chest for recording of RR and inserted subdermally on left side of chest for ECG recording.

The EEG records of all animals were visually examined in bipolar montage. The reviewer was asked to describe the background activity, possible normal variants and changes before euthanasia. After gas application the time of first changes in EEG, the duration of changes and the point of disappearance of EEG activity were noted. In addition, breathing pattern and its duration as well as ECG were evaluated. Reviewer (JJ) was blinded during the EEG, ECG and respiratory analysis (no information which gas was used for euthanasia in particular animal).

Electroencephalography, recorded before gas application, was similar in all groups and consisted of low activity (delta and theta) and low amplitude. Frequent finding was sleep spindles, breathing, ECG and other electrical artifacts.

At and post-euthanasia treatments

When the pre-euthanasia recordings were performed gas was let into the euthanasia chamber and immediately the continuous recording of BAER, EEG and ECG was started. These recordings were continued until no brain activity was recognised and until the heart arrest (asystole) was detected. Summary of the results is given in Table 1.

Table 1. Summary of EEG, BAER, respiration and ECG results

Group name	EEG		BAER	
	normal	absent	normal	absent
Exhaust	42±10 a	86±35ab	63±19 ab	176±37 a
CO_2	39±10 a	75±28 a	45±16 a	183±19 ab
CO 4%	55±11 ab	190±81 bc	143±70 bc	390±139 bc
CO 2%	176±126 b	426±252 c	305±148 c	833±345 c
P group	<0.01	<0.0001	<0.0001	<0.0001

Group name	Respiration		ECG	
	normal	absent	normal	absent
Exhaust	42±17 ab	217±53 a	105±37 a	292±130 ab
CO_2	28±5 a	227±26 a	117±31 a	220±92 a
CO 4%	144±67 bc	477±151 b	219±100 ab	289±126 ab
CO 2%	331±191 c	901±293 b	481±184 b	682±333 b
P group	<0.0001	<0.0001	<0.001	<0.01

Data are in seconds (mean ± SD). Letters a and b mark a statistical difference ($P<0.05$) between the groups. The pair-wise comparison was made according to Siegel and Castellan (1988). Statistical analyses by Kruskal-Wallis ANOVA.

It is known from the previous studies that it takes about 5 minutes for mink to die from gas euthanasia. Therefore the post-euthanasia BAER recordings were set so that the multiple recordings of the same animal were performed in 30 second intervals. The interval of 30 seconds was chosen as it is approximately 300 clicks needed to make the BAER averaging measurement possible. If it was noted that animals dying is taking longer the multiple BAER recording time was extended to 60, 90, 120, 150 or 180 second intervals.

The evaluation of BAER recordings after experiment was performed by one of the investigators (SC) and investigator was blinded when evaluating the BAER recordings. The following time intervals were evaluated in every individual 31 mink: time period in seconds during which the BAER recording stayed unchanged in terms of latency and amplitude (period of normal BAER), time period in seconds during which the BAER recording changed in latency and/or amplitude (period of decline of BAER) and the end time in seconds when the BAER recording could not be recognized (time of BAER disappearance). The data are shown in the following tables.

The range of duration of normal BAER recording during euthanasia was 30–90 seconds (mean 63) in exhaust gases group, 30–60 seconds (mean 45) in CO_2 group, 60–240 seconds (mean 143) in CO 4% group and 120–540 seconds (mean 305) in CO 2% group. The range of duration of decline of BAER during euthanasia was 90–180 seconds (mean 112) in exhaust gases group, 90–180 seconds (mean 138) in CO_2 group, 120–420 seconds (mean 235) in CO 4% group and 330–1020 seconds (mean 528) in CO 2% group. The time point of BAER disappearance during euthanasia ranged between 120 and 240 seconds (mean 176) in exhaust gases group,

150–210 seconds (mean 183) in CO_2 group, 180–660 seconds (mean 390) in CO 4% group and 480–1380 seconds (mean 833) in CO 2% group.

Typical finding after gas application was burst suppression (25/31) in EEG traces, increased respiratory rate and amplitude (27/31) and decreased amplitude and frequency of ECG recording (19/31), arrhythmia (9/31) or asystole (7/31). Changes in EEG, respiration and heart activity appeared to occur earlier in engine exhaust gas (EEG: mean 42 seconds; respiration: mean 42 seconds; ECG: mean 105 seconds) and CO_2 groups (EEG: mean 39 seconds; respiration: mean 28 seconds; ECG: mean 117 seconds). In contrast, changes in 4% CO group (EEG: mean 55 seconds; respiration: mean 144 seconds; ECG: mean 215 seconds) and 2% CO group (EEG: mean 176 seconds; respiration: mean 331 seconds; ECG: mean 481 seconds) occurred later. Termination of EEG, breathing and ECG also was faster in the first two groups compared to CO groups (respectively mean EEG, respiration and ECG: 86; 217; 292 (engine gas), 75; 227; 220 (CO_2), 190; 477; 289 (4% CO) and 426; 901; 682 (2% CO)). One animal in 2% CO group (#33) had no cortical activity according on EEG, but was breathing, with auscultable heart sounds after removal from the euthanasia chamber after more than 20 minutes since gas application and was euthanized with intraperitoneal injection of Thiopental.

Sneezing or coughing of the animals was not observed during exposure to the studied gases. Furthermore, no signs of irritation on areas of eyes, nose, mouth or respiratory ducts were found. Nor did we see any signs of bleeding in those areas.

Discussion

The results of the current experiment indicate that the electrophysiological recordings are possible to perform in the mink in a similar way as in other animal species. If animal is adequately sedated recording is technically straightforward. All animals here were sufficiently anesthetized (Jepsen et al., 1981) as documented by absent corneal, palpebral and withdrawal reflexes in all animals and the vital parameters such as breathing and heart rate stayed in reference range.

The results of BAER, EEG, respiratory rate and ECG recording indicate that the results of the filtered exhaust gas (CO) and the cylinder CO_2 group are similar. The duration of the normal brain activity as noted by BAER and EEG recordings, the time period of decline of the brain function and the time point of disappearance of BAER and EEG within these two groups was very similar. The above-mentioned parameters in the cylinder CO 4% group were longer than in the filtered exhaust CO and the cylinder CO_2 groups. These parameters were the longest in the cylinder CO 2% group and not all animals were euthanized even after staying over 20 minutes within the chamber with cylinder CO 2% gas concentration.

Consciousness can be considered an animal's ability to feel emotions and control its voluntary mobility. An animal can be presumed to be unconscious when it loses its natural standing position, is not awake and does not show signs of positive or negative emotions such as fear and excitement (AVMA, 2007). The onset of uncon-

sciousness is very essential for humane euthanasia because it is an indication that the cerebral cortex has been rendered non-functional. 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 recording shows the activity of the cerebral cortex. EEG recording results indicate that the first changes in cortical function occurred on average within the first minute of gas euthanasia in filtered exhaust CO, cylinder CO_2 and cylinder CO 4% gas groups (mean duration of normal EEG recordings 42 and 39 and 55 seconds, respectively). In contrast, the changes in cortical activity of cylinder CO 2% group happened within the third minute. It is impossible to say for sure what the exact time point is when animal does not perceive surrounding world normally any more (unresponsive), but it most likely correlates with the onset of the first changes in EEG recording. Absent EEG indicates that animal is unconscious (AVMA, 2007). From our data we can conclude that state of total unconsciousness occurred within the second minute in filtered exhaust CO and cylinder CO_2 groups and within the fourth and seventh minute in cylinder CO 4% and cylinder CO 2% groups, respectively.

BAER recording reflects the integrity and function of the brainstem of the animal. Results of our experiment indicate that the first changes in brainstem function in animals occurred on average within the first and second minute of gas euthanasia in exhaust and the CO_2 gas groups (mean duration of normal BAER 63 and 45 seconds). During the next two minutes the gradual decline in brainstem function was observed in animals of filtered exhaust CO and the CO_2 gas groups and the brainstem death occurred during the fourth minute of the gas euthanasia. These changes were definite and irreversible. In contrast, normal BAER recordings were longer in the cylinder CO 4% and 2% groups. It took 3 to 5 minutes to visualize the first changes in BAER recordings on average, it took on average 4–10 minutes for BAER recording to disappear gradually and 6–13 minutes till the death of the brainstem could be seen on the BAER recordings in the cylinder CO groups. In addition, one animal was still alive after 23 minutes staying in the chamber with the 2% concentration of cylinder CO gases and needed to be euthanized by the overdose of the pentobarbital.

Changes in respiration occurred within the first minute in filtered exhaust CO and CO_2 groups and after third or fifth minute in cylinder CO groups, respectively. Heart function abnormalities started within the second minute in exhaust gas and CO_2 groups. Heart and respiratory arrest occurred within the fourth minute of euthanasia in exhaust gas and CO_2 groups.

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 takes always some time and may last longer than some other killing methods (AVMA, 2007; Makowska et al., 2009). Our electrophysiological recording results indicate that the death with the filtered exhaust CO and the CO_2 gases occurs quickly and in very comparable times. Animal becomes unconscious at the beginning of the second minute, brainstem functioning seizes within the second-third minute and animal death occurs after the fourth minute. The timing in cylinder CO groups looks less predictable and precise. The euthanasia with the cylinder CO 2% concentration

gases in some cases is too long and is most likely not suitable for the mink euthanasia in general. According to Von Oettingen (1944), the toxicity of CO will be increased by an increase in CO_2. While engine-produced CO always contains also CO_2 (2.6–8%), it may increase the effectiveness of killing process in the mink.

Two previous studies on actual mink euthanasia, namely those of Lamboy et al. (1985) and Hansen et al. (1991) are available in the literature. Experimental set-up in each study experiment has been different which causes difficulties for comparison of studies. In those two previous studies, animal was placed into the chamber not before proper level of gas concentration was achieved. In the present study, on the other hand, animal was first placed in the killing chamber and, thereafter, gas input was begun. This has to be done because of our present technical set-up. However, we know here the time lapse from the beginning of gas input since the proper level was achieved in the killing box. In the present study time lapse for filtered exhaust CO, cylinder CO_2, cylinder CO (4%) and cylinder CO (2%) was 30 sec, 60 sec, 15 sec and 8 sec, respectively. Thus, when comparing the dying process between different studies, this time lapse can be roughly considered. However, this comparison has to be done with certain caution because it is based on calculated figures. It is thus more like an estimate. Lamboy et al. (1985) found that EEG started to decrease 19 sec since mink was placed into filtered exhaust gas chamber and EEG was finally gone (isoelectric flat line) on average 36 sec from the beginning. In the present study, normal EEG was found until 42 sec and it was finally zero 86 sec from the placement of mink into chamber. When taking into account the time lapse from start of gas input to proper level in the present study, the numbers are 12 sec (42–30 sec=12 sec) and 56 sec (86–30 sec=56 sec). Our EEG values seem to be thus close to those of Lamboy et al. (1985) for filtered exhaust gases.

As concerns cylinder CO (4%), decreased EEG was found in the study of Lamboy et al. (1985) 13 sec from the beginning of gas administration and, correspondingly, 35 sec was taken for total isoelectric EEG flat line. In the present study, however, these times were essentially longer, i.e. 25 sec (55–30 sec=25 sec) and 175 sec (190–15 sec=175 sec), respectively. We cannot explain this difference between these two studies. Also Hansen et al. (1991) wondered about the short duration of cylinder CO euthanasia process found by Lamboy et al. (1985). The difference in the cylinder CO set-up between studies of Lamboy et al. (1985) and Hansen et al. (1991) was that the former used pure CO (99.5%) whereas the latter tested ready mixed CO (4%).

Hansen et al. (1991) did not measure EEG but evaluated dying process from the behaviour of the animals. Total time for dying by CO_2 and cylinder CO was found to be 152.9 sec and 215.1 sec, respectively. Our data on ceasing of respiration can be used to compare duration of dying processes between present study and that of Hansen et al. (1991). According to our results, respiration was finally gone (animal dead) at CO_2 administration in 167 sec (227–60 sec=167 sec). Corresponding value for cylinder CO was 274 sec. Here, CO_2 values from both studies are very close to each other. However, cylinder CO dying process was slower in our present study.

According to Cooper et al. (1998) exposure of non-sedated mink to CO_2 may cause sneezing and coughing. In the present study, in sedated mink, we did not see any signs of sneezing or coughing. Nor did we see such features in non-sedated mink

killed individually or in groups (Korhonen et al., 2011 b). According to our experiments, CO_2 as well as CO, cannot be considered aversive to mink. It is difficult to see that these gases could expose mink to high discomfort during euthanasia process. The present results did neither reveal any observable signs of irritation on areas of eyes, nose mouth or respiratory ducts. Effect of exposed gases was primarily and first seen in brain (EEG), leading to state of non-pain in proper time. Therefore, we considered that possible irritative or aversive effects of CO and CO_2 gases are slight and probably do not cause any actual negative welfare implications.

According to Korhonen et al. (2011 b) both filtered exhaust CO and cylinder CO are equally effective when killing non-sedated mink in groups. In the Netherlands, cylinder CO has been used for decades. Farming practice has shown that mink are effectively killed by cylinder CO (Korhonen et al., 2011 a). Also our study (Korhonen et al., 2011 b) showed that when CO concentration at the end of killing process is about 4–5%, mink can be properly killed. That study also agreed well with the present findings that CO concentration of 2% is too low for effective killing.

In terms of the total killing time of a group, all three studied gas methods, namely filtered exhaust CO, cylinder CO and cylinder CO_2, were found effective by Korhonen et al. (2011 b). However, other variables tempted to conclude that the most appropriate method was cylinder CO_2. In particular, the time required for the first and last animal to fall/lie down was very short with CO_2. Loss of movement coordination and a natural standing position is a clear sign of loss of consciousness (Hansen et al., 1991; AVMA, 2007). The short time before an animal falls down led the authors of that study to conclude that mink lose consciousness more rapidly with CO_2 than with CO. This conclusion is supported also by the present results, 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.

Our previous study (Korhonen et al., 2009) showed that electric shock is effective to kill farmed blue foxes. First brain (EEG) and heart (ECG) is affected but deeper part of brain, i.e. brainstem activity (BAER), will last longer. So, electricity first affects certain parts of brain structure and heart but deeper activity in the brainstem typically stays longest. The present results showed that studied gases in the mink first affect brain and brainstem, which was seen as loss of EEG and BAER and just thereafter respiration and heart rate in turn. While sensitivity to pain is essentially related to consciousness and function of brain (AVMA, 2007), tested gases can be considered to primarily – and effectively – lead to state of non-pain in the mink. They are also effective to kill the animal in proper time.

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Elektrofizjologiczne badania nad eutanazją norek (*Mustela vison*) za pomocą CO i CO_2

STRESZCZENIE

Celem badań było określenie momentu śmierci zwierzęcia i tempa zmian w funkcjonowaniu jego mózgu i serca. Cztery grupy samców norek (*Mustela vison*) standardowych w typie ciemnym poddano sedacji i uśmiercano za pomocą: przefiltrowanych spalin (stężenie CO w boksie do eutanazji 4%, 8 zwierząt), dwutlenku węgla (CO_2 z butli, stężenie w boksie do eutanazji 80%, 8 zwierząt), tlenku węgla (CO z butli, stężenie w boksie do eutanazji 4%, 9 zwierząt), tlenku węgla (CO z butli, stężenie w boksie do eutanazji 2%, 6 zwierząt). Odpowiedź pnia mózgu na bodziec akustyczny (BAER) oraz elektroencefalografię (EEG), elektrokardiografię (EKG) i częstość oddechów mierzono przed i podczas eutanazji. Po zastosowaniu gazu, średni czas pojawienia się spadku/zaniku odpowiedzi pnia mózgu wyniósł w grupach odpowiednio 112/176, 138/183, 235/390 i 528/833 sekund. Po zastosowaniu gazu, średni czas pojawienia się pierwszych zmian/braku EEG wyniósł w grupach odpowiednio 42/86, 39/75, 55/190 i 176/426 sekund. Po zastosowaniu gazu, średni czas pojawienia się pierwszych zmian oddechowych/braku oddechu wyniósł w grupach odpowiednio 42/217, 28/227, 144/477 i 331/901 sekund. Po zastosowaniu gazu, średni czas pojawienia się pierwszych zmian/braku EKG wyniósł w grupach odpowiednio 105/292, 117/220, 215/289 i 481/682 sekund. Uzyskane wyniki wskazują, że badane gazy w pierwszej kolejności zaatakowały mózg i pień mózgu (co objawiło się brakiem EEG i BAER), a zaraz potem oddychanie i tętno. Podczas gdy wrażliwość na ból jest zasadniczo związana ze świadomością i funkcjonowaniem mózgu, uważa się, że gazy przede wszystkim skutecznie wywołują stan braku bólu. W szczególności uśmiercanie za pomocą przefiltrowanego CO oraz CO_2 z butli następuje szybko i w porównywalnym czasie. Eutanazja za pomocą 2% CO z butli wydaje się zbyt długa i najprawdopodobniej nie nadaje się generalnie do uśmiercania norek. Podczas wystawienia zwierząt na działanie badanych gazów (CO i CO_2) nie zaobserwowano oznak wyraźnego podrażnienia czy awersji.