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Acute Inhalation of Generator Exhaust-Fumes May Impair Learning and Memory in Mice

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Abstract

The study assessed the effects of acute inhalation of generator exhaust fumes on the anxiety-associated learning and memory (L&M), and motor coordination in mice. Twenty-four mice were divided into 4 groups. First and second groups were tested for motor coordination (beam walk) and L&M (Elevated Plus Maze, EPM) respectively. Mice in the experimental groups were exposed to the generator fume for 30min., 1hr., and 2hrs respectively before the tests. In Day 1, experimental animals showed significant decrease in the mean TL (13.4 ± 1.5sec.) and an increase (73 ± 8.4sec.) at 30min. and 1hr respectively, when compared to their controls (28.6 ± 5.2sec.) and (21.8 ± 4.4sec.). In day 2, there were significant increase in the mean TLs during the 30min. (48.4 ± 12.8sec.) and 2hrs exposures (66.8 ± 15.3 sec.), when compared to their controls (13.6 ± 1.9sec.) and (22.6 ± 6.6sec.) respectively. The result suggests decreased ability of the exposed mice to learn and recall the new behaviour. There was significant increase in the mean %COHb level of the experimental group (2.06 ± 0.012%) when compared to the control (0.76 ± 0.089%). Exposure to CO from the generator exhaust fumes impaired learning and memory in mice.

Keywords: Carbon monoxide, Learning and Memory, Motor coordination, Elevated Plus Maze, Beam walk.

INTRODUCTION

Human beings live together in harmony with other living and non-living things. These relationships help to establish a balanced ecosystem in agreement with nature. However due to population growth, industrialisation and other human activities, the environment became saturated with diverse pollutants giving rise to pollution. Significant sources of air pollution in urban areas of developing countries like Nigeria may include but are not limited to emissions from motor vehicles (Mayer, 1999) and generator exhaust (Stanley *et al.*, 2010). Carbon monoxide is a major air pollutant both indoors (Ayodele, 2007) and outdoors (Ajayi and Dosunmo, 2002) because of increased use of biofuel as a major source of energy for cooking.

Gasoline and diesel fuels are mixtures of hydrocarbon compounds containing hydrogen and carbon atoms. In a "perfect" engine, oxygen (O_2) in the air would convert all the hydrogen

and carbon in the fuel to water and carbon dioxide (CO_2) respectively. In reality, the combustion process is not "perfect" therefore CO is produced instead of CO_2. Also, production of CO is subject to the engine maintenance and nature of the fuel used (United States Air and Radiation, 2011). Petrol produces 28 times more CO than diesel (Campbell, 2009).

Carbon monoxide (CO) is one of the most common and widely distributed air pollutants in the world. It is a colourless, odourless, and tasteless gas that is poorly soluble in H_2O and has a slightly lower density than air (United States Air Quality Criteria, 2000). Although CO is not among the normal respiratory gases, its physio-chemical similarities with O_2 allow it to be transported through the airways and across alveolo-capillary membranes in a similar way. Therefore, at rest, and without exogenous exposure, minute quantity (0.4 - 0.7%) of CO in form of carboxy-haemoglobin (COHb) is produced endogenously in the body from catabolism of Haemoglobin (Hb) and acts as physiologic smooth muscle relaxant, neurotransmitter and cytoprotector against oxidative stress (Ryter *et al.*, 2004; USEPA, 2011). However, high concentrations of CO due to exogenous exposure may result to avid attachment to Hb and impaired O_2 delivery to organs especially the highly metabolic ones like the brain and heart.

Neurological symptoms are the most frequent in any case of CO poisoning. Early symptoms of mild poisoning may include headache, dizziness, weakness, nausea, confusion, disorientation, and visual disturbances, while severe exposures may result in confusion, loss of consciousness, or death (Amitai *et al.*, 1998). Symptoms of chronic CO poisoning like chronic fatigue, affective conditions, emotional distress, memory deficits, and difficulty in walking are usually subtle and may be overlooked as part of daily life chores (Penney, 2008). This could be one of the many reasons why CO is considered "the silent killer". There could also be delayed manifestation of neuropsychiatric impairment days after poisoning has occurred (Lam *et al.,* 2004).

Carbon monoxide poisoning was defined as any case of accidental or deliberate exposure with a history suggestive of CO exposure and exhibiting symptoms and signs of CO intoxication as well as elevated blood %COHb levels. Carbon monoxide poisoning accounts for 50,000 emergency department visits per year in the U.S. and is the most common cause of death due to poisoning in the country (Hampson *et al.*, 1996). Even with the paucity of data (Ajayi, A. and Dosunmu, 2002) high amounts of CO were recorded in some cities in Nigeria. Ambient levels in Ibadan city alone were between 3 and 55 ppm (Sunny *et al.,* 2008). The average indoor CO values measured in Kano were between 4.93 μ/m^3 in the municipality and 5.49 μ/m^3 in Sabon Gari area of the state (Ayodele *et al.*, 2007). The above values are by far beyond the U.S. National Ambient Air Quality Standards (U.S. NAAQS, 2011).

There was paucity of reported data on the incidence of acute or that of chronic toxicity in Nigeria. The few suspected cases of acute CO poisoning brought to the hospitals were grossly underestimated (Augustine, 2002). This is because the symptoms of CO poisoning are general symptoms (Samuel *et al.*, 2007) and mimic that of other common illnesses like flu (U.S. NAAQS, 2011). The U.S. Consumer Product Safety Commission (CPSC) of 2002 stated that every house should have a CO alarm and every major road junction must have one CO monitoring station to monitor CO levels; however, this is not found in Nigeria. Some hospitals in Nigeria are poorly equipped with CO-oximeter to make diagnosis easier. Therefore, poisonings are grossly under-reported or frequently misdiagnosed as usual flu symptoms.

There is a global transition to cleaner sources of energy and less environmental pollution. However, in Nigeria there is still increased importation of gasoline-powered generators due to instability of the main power grid (Akinbami *et al.*, 2001). The use of portable generators in the U.S. and other developed countries is only circumstantial, during power outages caused by winter storms or during forest camping (Niel *et al.*, 2005); but in Nigeria it is the usual source of power.

Similar studies were lacking after searching the literature because most CO poisonings occur accidentally during fire out brakes or use of generators in poorly ventilated houses when the exhaust seeps into rooms where people sleep. Severe cases of CO toxicity led to death, however, mild to moderately cases present with clinical features similar to that of common flu. Because autopsies are not usually conducted to ascertain the cause of death, therefore most cases were not reported, but end up as mainstream news from media houses. The aim of the study was therefore to assess the effects of acute, mild, CO poisoning from generator exhaust fumes, that may not kill but can affect the functions of vital organs such as the brain in mice, since the research cannot be replicated on human subjects.

MATERIALS AND METHODS

Materials
Source of carbon monoxide: Gasoline powered generator (TIGER, TG950, 220v/240v) manufactured by Suzhou Tiger Power Machine Co., Ltd., China served as the source of CO.
Portable digital carbon monoxide gas analyser: The CO meter, Amprobe, CM100 was placed inside the gas chamber to record the dose of CO in parts per million (ppm). The dose of 100–150 ppm of CO exposure was maintained throughout the study by adjusting the positions and the direction of the exhaust fumes of the Generator depending on the wind direction.
Gas chamber: The gas chamber measures 150 x 100 x 100 cm (length x breadth x height). There was an open window measuring 30 x 40 cm that allows the fumes into the chamber and also partial ventilation to fresh air.

Methods
Animals: Twenty-four adult swiss albino mice weighing between 18-32 g were used for the study. Mice were maintained at room temperature, fed on standard feed, and allowed access to tap water *ad libitum*. They were allowed to acclimatize with the environment for at least two weeks before commencement of the study.
Grouping: Mice were randomly assigned into one of the four groups. The first group was tested for motor coordination using beam walk paradigm and the second group for learning and memory, using Elevated Plus Maze (EPM) paradigm. The 3rd and 4th groups were their controls. Female mice were tested without monitoring the oestrous cycle.

Exposure of mice to CO from the generator exhaust fumes: Animal cages of the experimental groups were placed inside the gas chamber. Mice in the experimental groups were directly exposed to the exhaust fumes of the generator through the window for 30 minutes, 1 hour and 2-hour periods respectively before the neurobehavioral tests. The exposure method was adopted from a study by Samuel and Micha, (2007) where human subjects were accidentally exposed to fumes of gasoline-powered generator (5 kW, 3000 U/min.) directly adjacent to a long stable where they were sleeping. The two control groups were however maintained on fresh room air.

Neurobehavioral Tests:
Assessment of motor coordination using the beam walk test: In the beam-walk test, mice were trained to traverse an elevated, narrow ruler beam (for training) or a round beam (for the test) to reach an enclosed escape goal box in a model described by Stanley, (2005). The latency and foot-slip measurements were taken as the mouse traversed the beam.

Assessment of learning and memory using the elevated plus maze test: For learning and memory, the EPM paradigm as described by Itoh *et al.*, (1990) was used. On the first day (D1/ acquisition test) a mouse was placed at the end of one of the open arms, facing away from the central platform. Latency for the mouse to enter one of the closed arms was recorded in seconds. Following entry into an arm, the mouse was allowed to explore the apparatus for 30 seconds. Twenty-four hours later, the second trial (retention/ recall test) was performed. The time taken for a mouse to move from the starting point of the open arm to any of the closed arms was measured (in seconds) to indicate learning (D1) and memory (D2) (Itoh *et al.,* 1990).

Determination of the blood carboxyhaemoglobin in mice: At the end of the neurobehavioral tests (which took 3 days), mice were sacrificed, and blood samples were collected in an EDTA bottles via cardiac puncture. The blood samples were later processed and used for estimation of the %COHb by means of spectrophotometric method (Ernest and Carol, 1984).

Data analysis: Data obtained from the study were expressed as means ± SEM. Student's t-test was used to compare the means between experimental and control groups, as well as between Day-1 and Day-2 of the groups. For all evaluations, values of p < 0.05 were considered to imply statistical significance. Microsoft Office Excel version 2007 and SPSS version 15.0 were employed in analysing the data.

RESULTS
Learning: When transfer latencies (TL) of the control and experimental groups were compared in the three exposure periods, only the 30 minutes and 1-hour exposures showed significant change with P values of 0.023 and 0.001 respectively, at P < 0.05. Although latency of the experimental group drops to 13.4 ± 1.5 seconds during the 30 minutes of CO exposure, that of 1 hour exposure period increased to 73 ± 8.3 seconds when compared to their respective controls (28.6 ± 5.2 sec.) and (21.8 ± 4.4 sec.) as observed in Figure 1.

Memory: There were significant increases in the TLs of the experimental group during the 30 minutes (48.2 ± 12.8 sec.) and 2 hours (66.8 ± 15.3 sec.) of exposure as compared to the controls (13.6 ± 1.9 sec.) and (22.6 ± 6.6 sec.), respectively (Fig. 2).

Motor coordination: There were no statistically significant differences between the control and experimental groups to suggest any motor coordination deficits in all the three periods of CO exposure (Fig. 3).

Blood Carboxy-Haemoglobin: There was significant (P = 0.0001) increase in the percentage COHb of the experimental group (2.06 ± 0.12) when compared to the control (0.78 ± 0.089) group (Table 1.0).
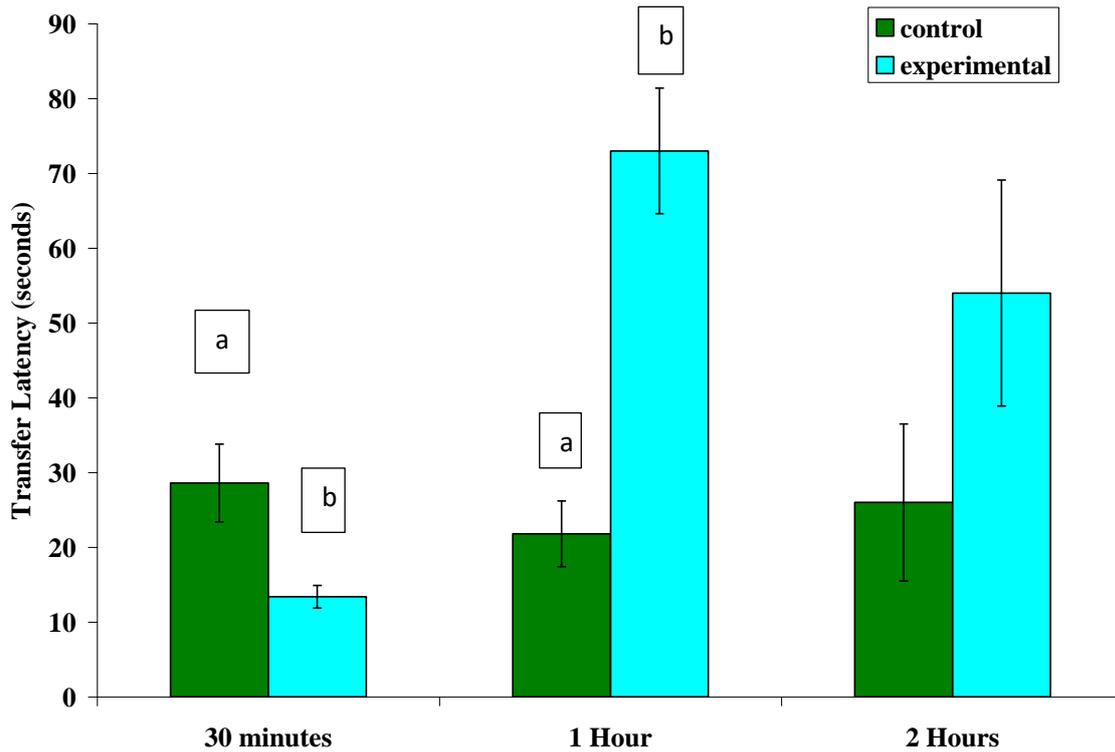
Figure 1: Transfer latencies of the control and experimental groups during the acquisition/learning test
[a, b] = values with different superscript letters indicate significance at $P < 0.05$ and its absence imply insignificance. n = 6
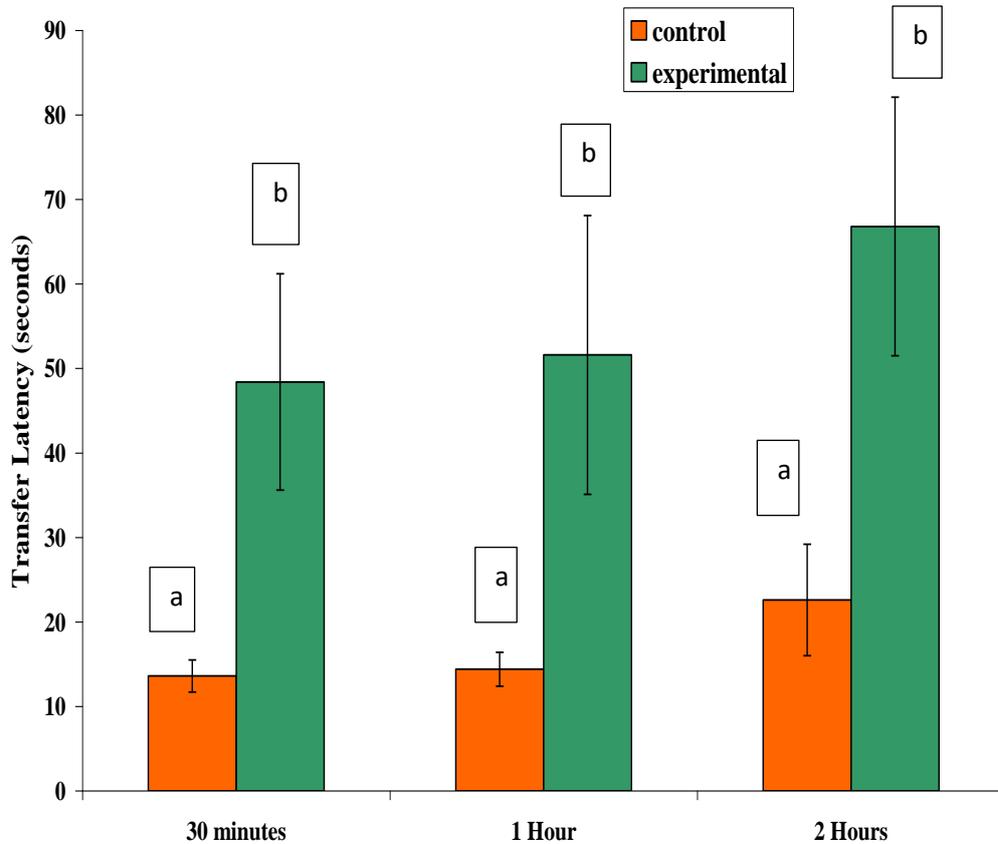

Figure 2: Transfer latencies of the control and experimental groups during the recall/memory task (Day-2).
[a, b] = values with different superscript letters indicate significance at $P < 0.05$ and its absence imply insignificance. n = 6
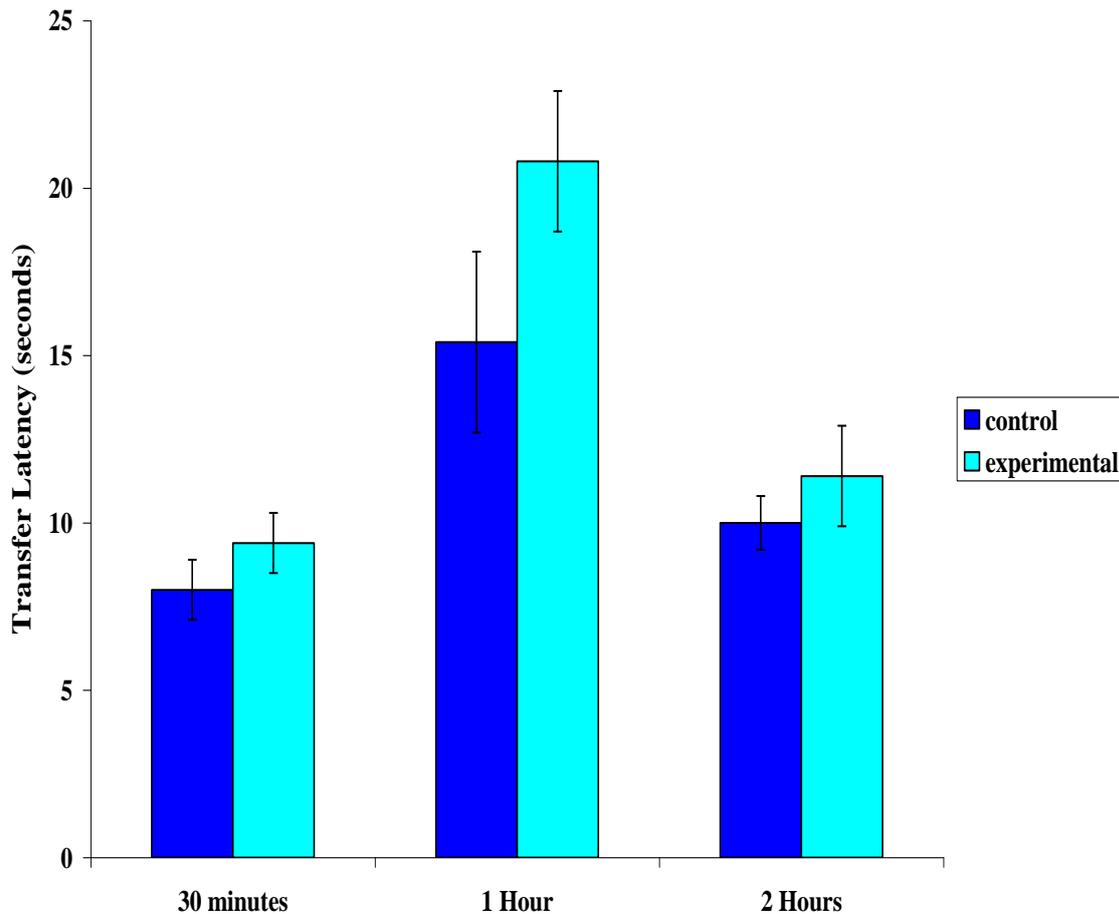
Figure 3: Latencies of the control and experimental groups during beam walk performance test.

Table 1: Percentage Carboxy-Hemoglobin in the control and experimental groups.

Groups	Percentage COHb in blood (Mean ± SEM)
Control	0.76 ± 0.089
Experimental	2.06 ± 0.012

DISCUSSION

Several studies in humans confirm neurotoxicity to be the most prominent symptoms in CO poisoning (Samuel and Micha, 2007; USEPA, 2011). Animal studies also suggest foetal susceptibility and the developing brain having the highest sensitivity among all other organs (Longo *et al.*, 1967). Significant increase in the TLs observed in the study suggest that CO exposure might have affected either the acquisition of the new behaviour, recall of the new behaviour or both. While significant decrease in the TLs may suggest that CO enhanced learning and or memory. Our findings revealed enhanced ability of mice to learn the new behaviour during the 30 minutes partial exposure to CO. This finding agrees with that of Boeihning et al., (2003) who reported that CO, together with NO act as gaseous neurotransmitters in the Central Nervous System (CNS) at physiological concentrations (Boeihning *et al.*, 2003), As the exposure increases, poisoning may occur (Mannaioni *et al.*, 2006). Karim *et al.*, (2001) had earlier established the role played by CO produced in the body as a retrograde synaptic messenger with a prominent position in the long-term potentiation (LTP) of certain areas of the brain. In a similar study CO produce long-term

enhancement when paired with low-frequency stimulation and results were found to be consistent with the hypothesis that NO and CO, either alone or in combination, serve as retrograde messengers that produce activity-dependent presynaptic enhancement during LTP (Zhuo *et al.*, 1993; Hawkins *et al.*, 1994).

Previous studies reported specific toxicity of CO on memory functions in animals and also delayed neuronal death in areas involved in memory process (Piantadosi *et al.*, 1997; Nabeshima *et al.*, 1991). Equally Katoh *et al.*, (1991) reported a 17% decrease in pyramidal cells in the CA1 region of mice, who received pure CO compared to normal controls. Our findings were in line with the above thus, suggesting significant deterioration in learning/ acquisition after 1 hour of partial CO exposure. Mereu *et al.*, (2000) associated the learning and memory deficits produced in rat offspring by a prenatal exposure model simulating the CO exposure observed in human cigarette smokers (150 ppm) with the functional changes observed at CA3-CA1 synapses in the hippocampus. Though 30 minutes of partial CO exposure enhanced the learning process, the same period of exposure produced significant deterioration in recall after 24 hours. It can therefore be deduced that CO exposure may impair recall in mice. Similarly, CO poisoned subjects were found to have impaired ability to remember new temporal, linguistic, and spatial information while previous knowledge was intact (Hopkins *et al.*, 1993). Short-term memory loss, lack of spontaneous movements and infarctions in bilateral hippocampi, temporal poles, occipital cortex, globus pallidus, cerebellum, and brainstem were apparent in an elderly man after deliberate CO poisoning. Although he was ambulatory six months later with normal communication; he suffered residual anterograde amnesia (Gottfried and Anjan, 2001). A case of delayed encephalopathy and cognitive sequelae were observed 4 weeks after acute CO poisoning, following which the patient regained some improved memory and other cognitive function after 4 weeks of treatment, including hyperbaric oxygen therapy (HBOT) (Tapeantong and Poungvarin, 2009). A significant deterioration of memory that was directly proportional to the duration of CO exposure was also observed. From the literature, the duration of exposure has a greater impact on the magnitude of CO patho-physiology than what is predicted based on the concentration of CO that was inspired (Fein *et al.*, 1980; Penney *et al.*, 2008).

Marginal increases in the TLs of the experimental groups in the Beam walk test, may suggest absence of any gross motor coordination deficits in all the three periods of CO exposure. However, previous studies have shown that gross affectation of movement is possible only at higher COHb level beyond 2%. Headaches, generalized weakness, fatigue and sleepiness were part of the vague symptomatology observed in human subjects with COHb levels below 20 % (Hampson *et al.*, 2008).

The amount of Haemoglobin (Hb) found in the blood samples were adequate (14 g/dl), and comparable in both groups. Therefore, difference in the percentage COHb found in the two groups cannot be attributed to the Hb content. Mean COHb level estimated was found to be higher in the exposed group than the control. Only a small amount of CO is formed in the body from endogenous production, larger percentage of the gas was from exogenous sources. The level of COHb found in the control group was comparable to that of endogenous production; however, that of experimental group was comparable to CO exposure of 15 ppm deduced from the Coburn-Forster-Kane (CFK) table (Supplementary file) (Coburn, 1965). Even though the dose of CO exposure in this study was in the range of 100 - 150 ppm, lower blood COHb levels were recorded. This might be because the method of exposure used in the study was partial, providing some fresh air ventilation to the experimental animals. Secondly, the prolonged time interval between exposure to CO and

blood collection may have also contributed to the low percentage COHb recorded when compared to the exposure dose.

CONCLUSION

In conclusion, CO at this range and period of exposure was found to adversely affect acquisition and recall but not motor coordination in mice. Our findings were in line with many previous studies, where acute CO exposure from fumes resulted in functional deficits in learning and memory. However, CO exposure at physiological level was beneficial to the learning process. Percentage COHb found in the exposed group was high, thus suggesting additional exogenous exposure. It is important to note that, various effects presented here might not be attributed to only CO, since several other contents of the generator exhaust fumes might perhaps be toxic.

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