RESEARCH ARTICLE

Spectrophotometric determination of carboxyhaemoglobin in a sample of automobile mechanics occupationally exposed to carbon monoxide

Catarina Dias [1]; Margarida Marques [1]; Vanessa Santos [1]; Ana Sofia Tavares[1]

[1] Escola Superior de Tecnologia da Saúde de Lisboa, ESTeSL, Instituto Politécnico de Lisboa (IPL), 1500-335 Lisboa, Portugal

Citation: Dias, C., Marques, M., Santos, V. & Tavares, A.S. (2018). Spectrophotometric determination of carboxyhaemoglobin in a sample of automobile mechanics occupationally exposed to carbon monoxide. *Res Net Health* 4, 1-7.

Received: 30th June 2017

Accepted: 24th July 2018

Published: 30th December 2018

Corresponding Author:
Ana Sofia Tavares
ana.tavares@estesl.ipl.pt

Abstract

Introduction: Carbon monoxide (CO) is a toxic, colourless gas that results from the incomplete combustion of coal and other petroleum-derived materials. Within anthropogenic sources of carbon monoxide emissions, air pollution from car emissions accounts for about 75% of the total air pollution present in atmosphere. Inhalation of this gas is considered to be potentially toxic to the body, resulting in a haemoglobin variant with reduced oxygen transport capacity, carboxyhaemoglobin (COHb). As the endogenous concentration of COHb in a healthy adult varies from 0.1% to 1.0%, higher values can lead to respiratory problems, visual perception impairment and manual dexterity, headache and nausea. The present study aimed to compare the amount of carboxyhaemoglobin present in a sample of 8 mechanics, working at automobile repair shops (test group), with the carboxyhaemoglobin present in a control group (16 non-mechanic individuals), via a spectrophotometric method, as well as to verify if the years of labour activity, along with the use of personal protective equipment (PPE) influences this parameter. **Methods:** A simple descriptive level II research study was developed to find relationships between variables. It is considered to be a case-control study and is further classified as analytical, observational, transversal and retrospective. **Results:** The results showed that the blood concentration of COHb (%COHb) in the test group was on average 0.653 ± 0.087% and in the control group, it was on average 0.477 ± 0.133%. Statistically significant differences were observed in relation to the carboxyhaemoglobin values between the test group and the control group ($p = 0.002$); however, no statistically significant differences were found between the years of work activity ($p = 0.711$) and use of PPE ($p = 0.392$) when compared to the carboxyhaemoglobin values of the test group. Although statistically significant differences were obtained in COHb values between the two groups, values higher than 1.0% in the test group (average of 0.653%) were not obtained. **Conclusions:** In this study, although no altered COHb values were found in the test group, new lines of research on this subject are recommended, aiming to broaden and deepen the field of knowledge, aiming at the protection of this type of workers.

Keywords: Carboxyhaemoglobin, Mechanics, Carbon monoxide, Occupational exposure.

Introduction

Haemoglobin (Hb) is the main component of blood, a pigmented protein that occupies about a third of the total volume of the cell and is responsible for its red colour. This protein is composed of four polypeptide chains and four Heme groups. There are several types of globin's, each with slight differences in its amino acid composition (Seeley, Stephens, & Tate, 2016). Meta-haemoglobin, sulfa-haemoglobin, and carboxyhaemoglobin are three abnormal haemoglobins. The increase of any of these haemoglobins in the bloodstream can be fatal (Ciesla, 2012).

Carbon monoxide (CO) is a toxic and colourless gas resulting from the incomplete combustion of coal and other petroleum-derived materials. The most common sources of CO emissions in the atmosphere have their origin, mainly from the gases released by motor vehicles, fires and industries (Topacoglu, Katsakoglou, & Ipekci, 2014). Active smoking is also another source of CO production

(Lacerda, Leroux, & Morata, 2005). This gas has a half-life of approximately 6 hours in the body (Barros, Schuck, Mana, Salicio, & Shimoya-bittencourt, 2012) and when aspirated, at the level of the pulmonary alveoli, it reversibly combines with haemoglobin to form carboxyhaemoglobin (COHb) (Fierro, O'Rourke, & Burgess, 2001). Since CO has about 250 times more affinity for haemoglobin than oxygen, when inhaled in large amounts, it causes tissue hypoxia due to the transformation of oxyhaemoglobin into carboxyhaemoglobin, a haemoglobin with reduced oxygen transport capacity (Buchelli et al., 2014).

It is estimated that the endogenous concentration of COHb in a healthy adult varies from 0.1 % to 1.0% (Malheiro, 1991), and in the absence of environmental exposure to CO, results in approximately 0.5 % COHb (Fernícola & Lima, 1979; Marshall, Kales, Christiani, & Goldman, 1995). However, in some haematological diseases such as sickle cell anaemia and thalassemia's, it can increase to values between 4% and 6% (Eyi, 2015). The formation of COHb and the appearance of clinical signs and symptoms depend mainly on the concentration of CO in the air, the time of exposure, the type of physical activity and the individual susceptibility. The main organs affected by its action are the brain and the heart, since they depend on a more demanding supply of oxygen (Malheiro, 1991).

The symptomatology of CO poisoning is quite unspecific, resembling other conditions such as a flu-like syndrome, gastroenteritis, hypertensive crisis or migraine (Barbosa, 2015). To make the diagnosis, a detailed clinical history associated with symptomatology and ancillary diagnostic must be done. The most frequent symptoms are headache (usually the first symptom), dizziness, fatigue, nausea, vomiting and drowsiness. High COHb concentration may confirm the diagnosis, but their values may not be related to the severity of the symptoms or may be normal if the patient has already been submitted to oxygen therapy or if the exposure has been ceased for long enough so that CO has been eliminated from the body. COHb reaches peak plasma levels after 1 hour of exposure and has a half-life of 4-5 hours (Barbosa, 2015).

Due to the diversity and the amplitude of the industrial processes and the energy needs of the population, we are therefore witnessing a large production of CO, which professionally occupies several workers daily. Among these, can be highlighted: workers in the metal and paper industry, refineries, synthetic metal production, firefighters, drivers, taxi drivers and automobile mechanics. Air pollution from car emissions has been shown to represent about 60% of all CO emissions (Fierro et al., 2001) and within a vehicle the average concentration of CO can be between 9 to 25 ppm. These values may be considered potentially toxic to the organism (Lacerda et al., 2005).

CO concentration levels in indoor environments may represent an even greater danger to occupational safety, since CO gas is heavier than air and thus can accumulate rapidly, even in well-ventilated areas (Fernícola & Lima, 1979). The level of CO concentration in the workplace environment is therefore a serious hazard to health and safety at work, especially in car repair shops, where employees are exposed daily to gases emitted by the internal combustion of engines of vehicles (Asimakopoulou, Kolaitis, & Founti, 2009).

Environmental monitoring, allows the assessment of occupational exposure and the compliance with the standards considered acceptable, taking into account the reference values or specific legislation, thus detecting if there is a risk situation for their health. These reference values are proposed by several international organizations, such as the World Health Organization (WHO), the American Institute of Occupational Safety and Health (NIOSH), American Society of Heating, Refrigerating and Air Conditioning Engineers (ASHRAE) and American Conference of Governmental Industrial Hygienists (ACGIH) (Silva, Robazzi & Terra, 2013). The National Institute for Occupational Safety and Health (NIOSH) allows exposure to 50 μL/L (50ppm) for 8 hours of work, which corresponds to 6-8% COHb; American Conference of Governmental Industrial Hygienists (ACGIH), recommends that exposure for 8 hours should never exceed 25 μL/L (25 ppm), which corresponds to a value of 3.5% COHb. According to the Environmental Protection

Agency (EPA) standards, the exposure should be 35 μL/L CO for 1 hour or 9 μL/L for 8 hours, to maintain COHb values < 2.0 % (Marshall et al., 1995).

In Portugal, the assessment of occupational exposure to chemical agents such as CO, also includes determination of the agent concentration in the workplace air and comparison with reference values representing acceptable exposure levels. The weighted average exposure limit value (ELV) is the weighted average concentration for a working day of 8 hours and a week of 40 hours, which is assumed that practically all workers can be exposed, day after day, without adverse effects ("Projeto de Norma Portuguesa NP 1796_2007, "2007). The weighted average ELV of 25 ppm for the specific case of CO is valid and is based on available information from industrial experience, experimental studies on animals and humans and, where possible, from all three sources. The ELV of the Portuguese Standard (NP) 1796: 2007, like other countries in the European Union, are based on the guidelines of ACGIH, in its 2006 edition ("Projeto de Norma Portuguesa NP 1796_2007, "2007).

At the respiratory level, as mentioned above, workers may be exposed to several workplace pollutants which include exposure to harmful particles, environments contaminated by vapours, hazardous gases or viruses, atmospheres whose oxygen concentrations are below or above the optimum levels or the combination of any of these situations. Respiratory protection masks, as personal protective equipment, are intended to protect the user's airways against contaminated atmospheres and with the potential to cause harmful effects on the health of exposed workers (ACT, 2015).

Several studies have been done with the main purpose of proving the effects of CO exposure on human health. Through these studies, most authors have concluded that elevated COHb levels can often be attributed to smoking, but another common cause is inhaled CO in the workplace. These situations should therefore be identified in order to implement protective measures and prevent CO poisoning. The study by Stewart et al. (1976), found that the mean COHb level in 55 non-smoking firefighters was 5.0%. The study conducted by Fernícola and Lima (1979), aimed to evaluate the degree of exposure to CO of the population of the city of São Paulo. The COHb values found in non-smoking traffic police were 2.10 ± 0.68%; Non-smoking bus drivers of 1.60 ± 0.46% and in the non-smoking control group was 0.80 ± 0.21%.

The study by Di Marco et al. (2005), showed that the main source of CO for participants not exposed to tobacco smoke were CO emissions from cars' leaks in traffic. In the study by González, Rivero and Marrero (2006), a group of 50 urban public transport drivers (non-smokers) and a group of 15 subjects who were not occupationally exposed to CO (non-smokers) were evaluated. The results showed that the mean level of COHb for exposed individuals was 3.20 ± 2.13% with symptoms of headache, fatigue and irritability and for the group not exposed, the mean level was 1.31 ± 0.74%. The study also revealed that the years of labour work of transport drivers did not influence the concentration of COHb.

In the investigation performed by Asimakopoulou et al. (2009), which aimed to compare levels of CO concentration in car repair shops with current occupational health legislation, it has been shown that CO levels indoors can pose a serious safety hazard in the job. In the study by Topacoglu et al. (2014), the objective was to compare COHb levels at the beginning of the working day with COHb levels at the end of the working day. The COHb values increased from 2.1 ± 2.0% to 5.2 ± 3.3%. The author concluded that the vehicle's high exhaust emissions have an impact on employee COHb levels. The study carried out by Salicio et al. (2016), aimed to analyse the exhaled CO and COHb in elderly practicing outdoor exercises and relating it to pollution. In this study, 118 elderly people, over 60 years old, of both sexes, exercising, healthy and non-smokers participated. In the study, mean levels of COHb were 0.43%, and no difference was found when compared to sex, age and old smoking habits.

Considering the state of art in this issue, the present study aimed to compare the amount of carboxyhaemoglobin (COHb) present in a sample of 8 mechanics, working at automobile repair shops (test group), with the carboxyhaemoglobin present in a control group (16 non-mechanic individuals), via a spectrophotometric method, as well as to verify if the years of labour activity, along with the use of personal protective equipment (PPE) influences this parameter.

Materials and Methods

Study design and participants

According to Fortin (2000), a simple descriptive level II research study was developed. It is still classified as a case-control study, observational, transversal and analytical. The study population consists of, males individuals, whose age range is between 21 and 70 years. For the sample selection, the non-probabilistic sampling method for convenience was used. The sample consisted of 8 participants in the test group and 16 in the control group. As inclusion criteria for this study, it was defined, for the test group, that the individuals must had to be over 18 years of age; being mechanics, working in car repair shops indoors and for the control group, individuals who did not work in a workshop setting. As exclusion criteria for this study, it was defined, the exclusion of individuals with haematological diseases and individuals with smoking habits.

Ethical considerations

In order to carry out this research, and with the final aim of using and presenting certain clinical data concerning the participating members of the study population, volunteers were proposed to read a free and informed consent and signing a declaration, in accordance with "The Helsinki Declaration of the Medical Association". In this way, all data collected through the survey completed by the participants was confidential, and all elements were anonymised. Participants were informed of the whole research process as they were given a full explanation of the nature and objectives of the study and given the possibility of clarifying all aspects.

Data collection

The data collection necessary for the execution of this study was carried out in the workplace of the test group and in the respective dwellings of the control group, and it was divided into two phases: in a first phase, participants completed a survey that allowed to verify the inclusion and exclusion criteria proposed in the study, as well as the reading and signing of informed consent. In the second phase of data collection, blood was collected in K3 EDTA tubes. The blood collection in the test group was performed at the end of the afternoon, after the 8 hours of work. According to Siqueira et al (2011), the spectrophotometric methods are the most used for their low cost, speed and simplicity so in this investigation, the spectrophotometric method chosen for the determination of COHb was that of Beutler and West (1984). This method is based on producing a mixture of two pigments by reducing oxyhaemoglobin and methaemoglobin with sodium hydrosulfite. Pigment absorptions are measured at two wavelengths at 420 and 432 nm (Beutler & West, 1984).

Data analysis

Data analyses were performed using the IBM Statistical Package for the Social Sciences (SPSS for Windows v. 23). Descriptive statistics including frequency distribution were developed. After the data normality check, Spearman coefficients were used to correlate the carboxyhemoglobin values with the years of labor activity. The Mann-Whitney test was used to verify whether there were significant differences in carboxyhemoglobin values between the exposed and the control groups, as well as to determine if there were significant differences in the carboxyhemoglobin values between the subjects who used and those who did not use PPE during working hours. Differences were considered significant if the p value was < 0.05.

Results and Discussion

As for the mean values of percentage of COHb (% COHb) in blood, in the test group an average of 0.653 ± 0.087% was obtained, while in the control group it was 0.477 ± 0.133%. Considering only the test group, regarding the frequency of declared use of PPE, 62.5% of individuals use PPE during working hours, while the remaining 37.5% do not. Table 1 summarizes the results obtained, regarding the descriptive statistics, for the remaining variables under study.

Table 1: Descriptive statistics of the variables under study.

Variable	N	Minimum	Maximum	Average	Standard deviation
Age[#] – Total sample	24	21	60	45.0	12.6
Age[#] – Test group	8	21	55	44.8	13.5
Age[#] – Control group	16	22	60	45.2	12.6
COHb Sat. %	24	0.273	0.805	0.536	0.145
Years of work activity[§]	8	1	40	16.3	15.2

[#]In years, [§]of the test group

The results showed that no statistically significant correlations were found between years of labour activity of the car mechanics and %COHb values ($p = 0.711$). When comparing the medians of %COHb values between the individuals who use and those who do not use PPE, it can be seen that the reported use of PPE in the test group had no influence on blood %COHb values ($p = 0.393$).

When comparing the medians of the %COHb values of the test group with the control group, it was showed that the medians of the two samples were not identical, and there were statistically significant differences between them ($p = 0.002$), which means that in the test group, blood %COHb values are significantly higher than the values obtained for the control group.

In order to assess whether there were significant changes in %COHb values in blood samples from automobile mechanics and the control group, twenty-six individuals completed a questionnaire. At the same time, a blood sample was collected to assess the levels of %COHb. The study population was exclusively male; due the fact that car mechanics is a profession whose jobs are mostly occupied by men. Since it was obtained samples from only males in the test group, it was opted for the control group, to follow the same rule, so all the samples analysed in this study are collected from males.

According to Fernícola and Lima (1979), the levels of CO concentration in closed environments may represent an even greater danger to the participation of mechanics in closed-car repair shops. CO gas is heavier than air, so it can accumulate rapidly even in well-ventilated areas. On the other hand, the participation of individuals with haematological diseases was defined as an exclusion criterion, since Eyi (2015) states that this type of pathologies may influence COHb levels, as well as the exclusion criteria for the participation of individuals with smoking habits, since according to Bain et al. (2016), these habits can influence the concentration of COHb with values up to 10% in smokers. After the analysis of the surveys, 2 individuals were excluded, due the fact they fulfilled the exclusion criterion.

In order to clarify the hypotheses of the study and to fulfil the proposed objectives, after performing the statistical analysis of the twenty-four valid samples, it was possible to verify that there were significant changes in the %COHb values between the test group and the control group. The mean of COHb concentration for the test group was 0.653 ± 0.087%, while that for the control group was 0.477 ± 0.133%. Results showed too, that the values of COHb in blood were significantly higher in the test group than the values obtained in the control group ($p = 0.002$).

In all of the studies, previously mentioned, although they had a different study population, they showed similar results with the present investigation, since in all of them there were increased values of %COHb, resulting from exposure to CO. Despite this similarity, the COHb values obtained in the present study were not as high as in the previously mentioned studies, since the mean in both the test and control groups was less than 1.0%. The study of Marshall et al. (1995), corroborates the values obtained in the control group (0.477 ± 0.133%), since it states that endogenous Heme catabolism, in the absence of exposure to CO, results in ~ 0.5% COHb. In the study of Salicio et al. (2016), in the elderly population, mean values of COHb of 0.43% were also obtained. Although the values obtained in the test group (0.653 ± 0.087%), were less than 1.0%, according to Lafontaine, cited by Fernícola and Lima (1979), the presence of COHb between 0.5 and 0.8% may correspond to a continues exposure to 5 ppm CO in the air, or an 8-hour exposure to 7 ppm or one hour at 20 ppm. These values of CO exposure are in accordance with the ELV of the Portuguese Standard (NP) 1796:2007, which states that the ELV-weighted average is 25 ppm for the specific case of CO in a working day of 8 hours and a week of 40 hours. According to this, it may be considered that all participants in the test group would be exposed to acceptable CO exposure values without adverse health effects.

It was also compared the years of labour activity with the %COHb values of the test group. Findings showed that there is no correlation between the two variables, years of labour activity and COHb levels (p = 0.711). The results obtained are consistent with the study by González et al. (2006), which showed that the years of work activity in public transport drivers do not influence the COHb concentration on blood. These results may be due to the fact that CO is not a cumulative toxicant and COHb is totally dissociable, once exposure to CO has ceased and Hb will revert to oxyhaemoglobin (Fierro et al., 2001). Considering that only a small number of participants in the test group stated that they used PPE, it was not possible to draw conclusions about the influence of the use of PPE on the COHb values.

Given the scarce literature on CO exposure and COHb determination in workers occupationally exposed to CO, such as mechanics who work in auto repair shops, it is recommended that new studies can address the present theme, aiming to broaden the field of knowledge and aiming to protect the population and workers. Therefore, new studies are recommended with the analysis of a larger number of mechanics of cars occupationally exposed to CO, starting for an experimental study that allows performing several measurements throughout the working day relating to different degrees of exposure to CO.

Conclusions

In this study the levels of %COHb in blood are determined in automobile mechanics. Knowing that the exposure to this gas (CO) is the origin of the onset of COHb and, in high concentrations, can be fatal, this has led to the interest of deepening this issue with regard to an occupational exposure to CO. Some obstacles were encountered during the study process, such as the difficulty in finding individuals who were willing to participate in the study, or those who accepted to participated, did not complete the inclusion criteria and for this same reason, had to be rejected. As causes of error, there is a possible adulteration of information collected through the surveys, since Edwards (1982) states the tendency to respond in a socially desirable sense.

This research is of great importance, since it gave too, the opportunity for participants to know the symptomatology associated with increased levels of COHb in the blood, such as the risks related to exposure to carbon monoxide. However, a larger study involving a greater number of individuals is necessary, so that the results of the sample fit the reality of the population. It may be important to correlate COHb levels with other pro-inflammatory biomarkers, so that general health status may be better related to the COHb differences found. It is hoped that the disclosure of this research will alert to the risks of exposure to carbon monoxide, in order to sensitize both the general population and the occupationally exposed population.

References

Asimakopoulou, E., Kolaitis, D.I. & Founti, M.A. (2009). CO dispersion in a car-repair shop: an experimental and CFD Modelling Study. *Seventh International Conference on CFD in the Minerals and Process Industries*. Melbourne: CSIRO.

Bain, B.J., Bates, I. & Laffan, M. (2016). *Dacie and Lewis practical haematology* (12th ed.). London: Elsevier.

Barbosa, M.M. (2015). *Tratamento das intoxicações pelo monóxido de carbono*. Master thesis in Medicine, Faculty of Medicine, University of Porto. Porto: UP.

Barros, S., Schuck, C.P., Mana, V.M., Salicio, M.A. & Shimoya-bittencourt, W. (2012). Avaliação da concentração de monóxido de carbono em estudantes universitários da área da saúde. *Journal health science institute* 30(4)*:* 399–405.

Beutler, E. & West, C. (1984). Simplified determination of carboxyhemoglobin. *Clinical chemistry*, 30(6): 871–874.

Buchelli-Ramirez, H., Fernández-Alvarez, R., Rubinos-Cuadrado, G., Martinez-Gonzalez, C., Rodriguez-Jerez, F. & Casan-Clara, P. (2014). Elevated carboxyhemoglobin: Sources of carbon conoxide exposure. *Archivos de bronconeumología (English edition)*, 50(11): 465–468.

Ciesla, B. (2012). *Hematology in Practice*. (2nd ed.). Philadelphia: E.A Davis.

Di Marco, G.S., Kephalopoulos, S., Ruuskanen, J. & Jantunen, M. (2005). Personal carbon monoxide exposure in Helsinki, Finland. *Atmospheric environment* 39(15): 2697-2707.

Edwards, A.L. (1982). *The social desirability variables in personality assessment and research*. Westport: Greenwood Press.

Eyi, Y.E. (2015). Endogenous and exogenous factors affecting the levels of carboxyhemoglobin. *American journal of emergency medicine* 33(9), 1309–1310.

Fernícola, N.A.G.G. & Lima, E.R. (1979). Avaliação do grau de exposição de amostras populacionais de São Paulo (Brasil) ao monóxido de carbono. *Revista de Saúde Pública* 13(2), 151-158.

Fierro, M.A., O'Rourke, M.K. & Burgess, J.L. (2001). *Adverse health effects of exposure to ambient carbon monoxide*. Tucson: The University of Arizona, College of Public Health.

Fortin, M.F. (2000). *O Processo de Investigação - Da concepção à realização*. Loures: Lusodidacta.

Gonçalves, N., Gaspar, N., Sequeira, R., Viegas, S. & Albuquerque, P. (2009). Exposição ocupacional a poluentes nos parques de estacionamento subterrâneos da Grande Lisboa. Lisboa: ESTeSL - Air in Route Report.

González, S., Rivero, E. & Marrero, S. (2006). Concentración de carboxihemoglobina en transportistas del sector urbano. *Informed* 8(10): 471–478.

Lacerda, A., Leroux, T. & Morata, T. (2005). Ototoxic effects of carbon monoxide exposure: a review. *Pró-Fono - Revista de Atualização Científica*, 17(3): 403–412.

Malheiro, A. (1991). *Determinação espectrofotométrica da carboxiemoglobinemia em indivíduos expostos ocupacionalmente ao monóxido de carbono.* Master thesis in Toxicologic analysis. São Paulo: University of São Paulo

Marshall, D., Kales, N., Christiani, D.C. & Goldman, R.H. (1995). Are reference intervals for carboxyhemoglobin appropriate? A survey of Boston area laboratories. *Clinical chemistry* 41(10): 1434-1438.

Projecto de Norma Portuguesa prNP 1796_2007. (2007). *Segurança e Saúde do Trabalho: Valores limite de exposição profissional a agentes químicos.* Caparica: Instituto Português da Qualidade.

ACT. (2015). *Riscos químicos - Guia de seleção de aparelhos de proteção respiratória.* Caparica: Instituto Português da Qualidade.

Salicio, M.A., Mana, V.A.M., Fett, W.C.R., Gomes, L.T. & Botelho, C. (2016). Variáveis ambientais e níveis de monóxido de carbono exalado e carboxihemoglobina em idosos praticantes de exercício. *Ciência e Saúde Coletiva* 21(4), 1023–1032.

Seeley, R.R., Stephens, T.D. & Tate, P. (2016). *Anatomia & Fisiologia*. São Paulo: Mc Graw-Hill.

Silva, L.A., Robazzi, M.L.C. & Terra, F.S. (2013). Asociación entre accidentes de trabajo y los niveles de carboxihemoglobina en trabajadores moto-taxistas. *Revista Latino-Americana de Enfermagem* 21(5): 1-8.

Siqueira, M.E., Martins, I., Costa, A.C., Andrade, E.L., Esteves, M.T. & Lima, S.A. (1997). *Revista de Saúde Pública* 31(6), 618–623.

Stewart, R.D., Stewart, R.S., Stamm, W. & Seelen, R.P. (1976). Rapid estimation of carboxyhemoglobin level in fire fighters. *JAMA* 235(4), 390–392.

Topacoglu, H., Katsakoglou, S. & Ipekci, A. (2014). Effect of exhaust emissions on carbon monoxide levels in employees working at indoor car wash facilities. *Hippokratia* 18(1), 37–39.