D. Adamović*

OCCUPATIONAL SAFETY IN THE MEDICAL LABORATORY AND FORMALDEHYDE EXPOSURE

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SUMMARY: Chemical hazards in their various forms deteriorate the quality of air in the work environment. The way of introduction of toxicants into the human body and the transformation pathways are the main factors that influence the realization of harmful effects. Due to the high prevalence in various industries, the attention of occupational professionals working in the quality of the working environment has been directed towards formaldehyde, over the last decades. The paper presents the essential characteristics of formaldehyde and the health effects caused by exposure to different concentration levels of this hazard. Emphasis is put on employees in medical laboratories where formaldehyde is used as a disinfectant and agent for the conservation of specimens in anatomy laboratories. The experimental part of the work was carried out in the laboratories of the Department of Anatomy, where FA concentration levels were monitored in different rooms to evaluate the exposure of employees.

Key words: *chemical hazards, formaldehyde, occupational exposure, medical laboratory*

INTRODUCTION

Chemical hazards are substances which may cause harm to human health and safety, because of its characteristics. Chemical substances can take a variety of forms. They can be in the form of solids, liquids, dust, vapours, gases, fibres, mists and fumes. The structure and phisico-chemical characteristics of a substance determine how it enters the human body, while the concentration levels of toxicants and the length of exposure determine the level of future consequences *(Gochfeld, Laumbach, 2017)*.

In the occupational settings, inhalation is the most important way of entry of chemical agents into the human body, followed by contact with skin and subsequent percutaneous absorption. Even though the gastrointestinal tract is a potential site of absorption, the ingestion of significant amounts of chemicals is rare in the occupational settings. The respiratory tract is exposed to chemicals in the inhaled air *(Montano, 2014, Anderson, Meade, 2014)*. The two dominant factors that determine the tissue responses to chemicals are the anatomy of the respiratory tract and the physico chemical nature of the material.

The removal rate of gases from the airstream during inhalation mostly depends for the most part on the water solubility of the gas. Highly water-soluble gases such as formaldehyde, ammonia, hydrogen chloride, and hydrogen fluoride dissolve readily in the moisture related with the mucous coating of the nasopharyngeal region, irritating those sites. At high concentration levels, some of the gas will not be absorbed in the upper respiratory sections, and amounts sufficient to reach the alveoli can cause severe irritation and pulmonary oedema *(Hathaway, Proctor, 2004)*.

*Dragan Adamović, Ph. D., (draganadamovic@uns.ac.rs), University of Novi Sad, Faculty of Technical Sciences, Dositeja Obradovića 6, 21000 Novi Sad, Serbia.

Comparatively insoluble gases such as nitrogen dioxide and phosgene are not removed by the moisture in the upper respiratory tract and can easily reach the alveoli. Substances of intermediate solubility such as chlorine can irritate points all along the respiratory tract.

The hazard or risk of a substance is the probability that it will cause injury in a given environment or situation. The danger of a substance depends on several factors, including its toxicity, way of absorption, metabolism and the way of excretion from the body; rapidity of action, its warning properties and it's potential for fire and explosion.

Over the last few decades, various epidemiological studies have revealed an increased risk of cancer development among workers exposed to formaldehyde (FA), namely nasopharyngeal cancer and myeloid leukaemia *(Schwilk et al., 2010, Zhang, 2010)*. Based on these findings, plus supporting evidence from animal studies and data on mechanisms of carcinogenesis, FA was classified as a human carcinogen *(Swenberg et al., 2013, Tang et al., 2009)*. FA is a high-volume production compound manufactured worldwide with a multiple ranges of applications.

Formaldehyde is a flammable, colourless gas with a strong pungent odour *(Abdollahi, Hosseini, 2014)*. In industrial conditions FA is synthesised by the methanol oxidation. FA reacts with strong oxidisers, alkalis, acids, phenols, and urea. It is between 25 the most abundantly produced chemicals in the world and it is used in the manufacture of plastics, resins, and urea formaldehyde foam insulation *(Wolverton et al., 1984)*. FA containing resins are used in the manufacture of a wide variety of organic products, explosives, artificial silk, and dyes. In the agricultural industry, FA has been used as a fumigant, an insecticide, a germicide and fungicide for plants, and in the manufacture of slow-release fertilisers *(Agarwal et al., 2015)*. FA is found in construction materials such as plywood adhesives.

FA is an irritant of the eyes and respiratory tract. It causes both primary irritation and sensitization dermatitis. At high levels FA is carcinogenic in experimental animals and, although results are unclear in humans, it is considered a suspected human carcinogen.

It has been used as an embalming fluid widely used in anatomy laboratories. Cadavers in anatomy laboratories are usually preserved by formalin, an embalming fluid which contains FA as a principal component. The FA tends to polymerize at high concentrations, and after long storage periods, for the above mentioned reasons it is often used commercially as a 37 %-40 % water solution of FA known under the name formalin *(Swami et al., 2016)*.

A major route of FA exposure for the general population is inhalation of indoor air *(Naya, Nakanishi, 2005)*; releases of FA from new or recently installed building materials and furnishings may account for most of the exposure.

Due to its extreme reactivity, FA is classified as both a potent irritant and sensitiser and is intensely irritating to mucous membranes. Mild eye irritation with lacrimation and other transient symptoms of mucous membrane irritation have been observed in some persons at concentrations of 0.1–0.3 ppm. Its presence is easily felt even in concentrations well below 1ppm *(Arts et al., 2005)*. Many published studies have also shown the odour threshold to be well below 1 ppm (odour threshold is 0.5 to 1.0 ppm). For sensitised persons, an odour is not an adequate indicator of FA presence and may not provide reliable warning of hazardous concentrations because odour adaptation can occur *(Wolkoff, 2013)*. For most people, however, a tingling sensation in the eyes, nose, and posterior pharynx is not experienced until 2–3 ppm. Some tolerance occurs, so that repeated 8-hour exposures at this level are possible. At 4–5 ppm irritation of mucous membranes increases and lacrimation becomes evident. Some may tolerate this level for short periods, but after 30 minutes, the discomfort becomes quite pronounced.

Concentrations of 10 ppm can be withstood for only a few minutes; profuse lacrimation occurs in all subjects, even those acclimated to lower levels. Between 10 and 20 ppm, it becomes challenging to take a normal breath; burning of the nose and throat becomes more severe and extends to the trachea, producing cough. On cessation of exposure, the lacrimation subsides promptly, but the nasal and respiratory irritation may persist for about an hour. It is not known at which levels severe inflammation of the bronchi and lower respiratory tract would occur in humans; it is expected that 5- or 10- minute exposures to levels of 50–100 ppm would cause severe injury. Acute irritation of the human respiratory tract from inhalation of high levels of formaldehyde has caused pulmonary oedema, pneumonitis, and death *(Hathaway, Proctor, 2004)*.

Occupational exposure to FA can occur during its production and during its use in the production of end products, in the textile industry, in the building materials industry, and in laboratories. In the European Union, the number of workers exposed to FA above the background level is calculated to be 1.7 million *(Dugheri et al., 2018)*. Although most exposed workers are foreseeably engaged in chemical and plastics factories, the highest mean levels of exposure were actually recorded in the health-care sector *(Tang et al., 2009)*. Health care professionals may be exposed to FA vapours during preparation, administration, and/or clean-up of various medicines. Pathologists, histology technicians, morticians, and teachers and students who handle preserved specimens may also be exposed.

Criteria referred to as legal standards or as recommendation standards are established by several organizations in the world. For example, The US Occupational Safety and Health Administration (OSHA) has established limits for the amount of FA that workers can be exposed to at their place of work. At present, the limit is at 0.75 ppm on average over an 8-hour workday (8h TWA time-weighted averages). The highest concentration level that a worker can be exposed to is 2 ppm, and that can only occur over 15 minutes (STEL) *(Golden, Valentini, 2014, OSHA, 2016)*. While the United Kingdom stands at 2 ppm for both TWA and STEL, the American Conference of Government Industrial Hygienists (ACGIH) has set a threshold limit value – ceiling (TLV-C) of 0.3 ppm *(Bono et al., 2012)*. NIOSH's Immediately Dangerous to Life or Health is 20 ppm for FA *(U.S. Department…, 1999)*. An 8-hour TWA is the average exposure concentration over the course of an 8-hour workday. A short term exposure limit (STEL) is defined by ACGIH as the concentration to which workers can be exposed continuously for a short period, usually 15 minutes, without suffering from irritation, chronic or irreversible damage, or narcosis. A TLV-C (threshold limit value – ceiling) is a concentration that should not be exceeded during any part of the working exposure.

Chronic exposure to low and sub low concentrations of formaldehyde may not be neglected, since these concentration levels are the initiators of serious illness for exposed workers. For the carcinogen effects there are no small safe doses, there is no threshold. Exposure standards cannot be used as a fine dividing line between a healthy and unhealthy workplace. It is supposed that the carcinogen effect occurs at any dose applied. This assumption is based on the knowledge of the biological evolution of cancer. All that is needed is a single molecule of a toxic substance to change a cell, giving it the possibility to develop itself into cancer.

Laboratory technicians, students and instructors are continuously exposed to FA during their work in the laboratories. Thus, the objectives of this paper were to measure the concentration levels of FA in the Anatomy laboratory of the Faculty of Medicine and to compare the findings with the standards. Other goals of this research were to provide relevant data about FA concentration levels during the standard teaching process and to assess the health risks of students, teaching and non-teaching staff residing on the premises of the Anatomy Department.

METHODS

The measurements of TWA concentration levels of FA were conducted at five locations inside the Anatomy Department in order to assess the exposure level of students and employees during the process of conducting pre-exam exercises for the final exam. In this period of the year, students, teaching and non-teaching staff are exposed to the highest concentration levels of FA due to the largest number of anatomical specimens that have been extracted from formalin. The task of this study was to get the most realistic image of workroom contamination levels, i.e. to make sure that the monitoring of FA concentration levels follows the Department's standard practice without letting the sampling procedure disrupt the normal work operations in any way whatsoever.

Air sampling was conducted in two classrooms, storage room, prepare room and break room in which the employees, professors and support technical staff reside during their breaks in the process of conducting classes. Figure 1 provides a schematic overview of rooms that were relevant for this study along with their measuring locations.

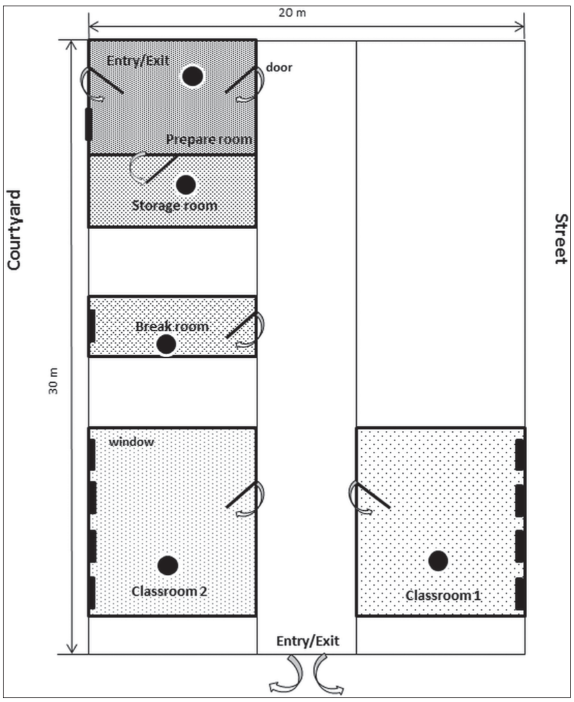

Figure 1. Schematic view of sampling points
Slika 1. Shematski prikaz točki uzorkovanja

Samples were collected with a constant flow pump using the air sampler PRO EKOS 401-x. The air was infiltrated through the Drechsel bottles with diffuser frit containing absorption solution for FA. Absorption solution was prepared by adding 9.5 ml of concentrated sulfuric acid and 0.5 ml of 1 % chromotropic acid into the Drechsel bottle. The air flow was set at 0.5 $dm^3 \cdot min^{-1}$. Samples were then analyzed in the same day, as the intensity of purple colour of the absorption solution remains stable only for a few hours.

Each sample was analyzed by adding 0.1 mL of 1 % chromotropic acid and 6 mL of concentrated H_2SO_4 to 4 mL of the sample solution. The solution was gently swirled to mix and left for 2–3 hours to cool down and to form a colored solution. A 2-cm cuvette was filled with the colored solution and placed in the spectrophotometer (Model Hach DR 5000- Germany), and the absorbance was read at 580 nm, against a blank solution (a solution containing 4-mL deionized water and that was treated as a sample). Finally, the FA content of the sample was determined using pre-prepared calibration curve.

The results are presented in Table 2 for the duration of time during which the samples were collected and as 8-h TWA.

Statistical analysis

The monitoring results were subjected to statistical analysis. The analysis of results was performed by using Statistica version 21, separately for each location. Conformity observed distributions with the theoretical normal distribution were analysed using the values of Skewness and Kurtosis and the homogeneity of variance for tested results of measurements was analysed using Levene's test. The results were analysed with analysis of variance (ANOVA) and post-hoc Tukey's test; $p=0.05$ was adopted to determine the significance of differences between results for the different locations.

The mutual influence of the different emission sources is calculated using the Pearson's correlation coefficients between the concentration levels of the FA in the various premises of the Department.

RESULTS AND DISCUSSION

In medical colleges, cadavers for anatomy laboratories are usually prepared by using FA as embalming fluid. In the presence of cadavers and during the process of dissection, FA vapors are emitted from the cadavers, resulting in the exposure of medical students and their instructors to elevated levels of FA in the laboratory.

During the experimental testings in this paper, 100 analyses were carried out in 5 measuring locations, during 20 work days. The values of the FA concentrations range from 0.01 ppm to a maximum concentration level of 6.60 ppm (Table 1). Outliers were not identified during the measurement campaign. The range of detected concentrations of FA during the campaign is shown in Figure 2.

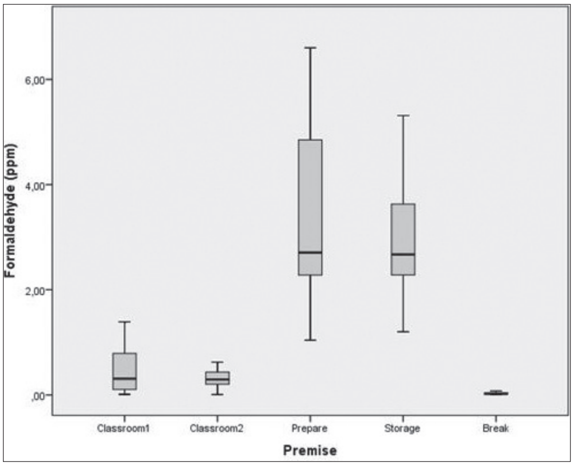

Figure 2. The range of detected concentrations of FA

Slika 2. Raspon utvrđenih koncentracija formaldehida

Preliminary statistical analysis indicates a normal distribution of measured values of FA concentration levels. The positive value of Skewness (1.23) in Table 1 indicates that most of the measurement results are below the mean value. The positive value of Kurtosis (0.64) suggests that the results of the measurements were accumulated around the mean value.

The results of the measurements of FA concentration levels during the campaign are shown in Table 1.

For the Classrom 1 (Table 1), the concentration of FA for the sampling duration ranged between 0.01 and 1.39 ppm with a mean of 0.48 ppm. The concentration range in the Classroom 2 is somewhat lower than in Classroom 1 and ranges from 0.01 to 0.62 with a mean of 0.48 ppm and 0.30 ppm, respectively. Detected FA concentrations in the selected period indicate that students, professors and laboratory technicians were exposed to concentration levels that were below the TWA values recommended by OSHA (0.75 ppm). Technical assistants were exposed to a significantly higher impact of the FA in the Prepare and Storage room. The values of FA concentrations in these rooms range from 1.04 to 6.60 ppm with a mean of 3.45 ppm and 2.91 ppm for a Prepare and Storage room, respectively. Detected values are significantly above the recommended values by OSHA. The lowest values of FA concentrations throughout the campaign were detected in the Break room. Values of FA concentrations were detected in the range from 0.01 to 0.08 ppm with a mean of 0.03 ppm.

The relationship between the concentration levels of FA in different premises of the Department was investigated using Pearson's correlation coefficients (Table 2). Preliminary analysis was carried out to prove the assumptions about the linearity and homogeneity of the variance. A

Table 1. Descriptive statistics and exposed group of employed workers

Tablica 1. Deskriptivna statistika i skupina izloženih zaposlenika

	Exposed group	**N**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
FAClassroom1	Students Professors Laboratory technicians	20	0.01	1.39	0.48	0.46
FAClassroom2	Students Professors Laboratory technicians	20	0.01	0.62	0.30	0.18
FAPrepare	Technical assistants	20	1.04	6.60	3.45	1.68
FAStorage	Technical assistants	20	1.20	5.31	2.91	1,09
FABreak	Cleaning staff and all other groups	20	0.01	0.08	0.03	0.02

strong positive correlation between FA concentration levels in Storage and Prepare room was calculated ($r = 0.772$; $n = 20$; $p < 0.01$), where high FA concentrations in Prepare room follow high concentration levels in the Storage room (Table 2). A strong positive correlation between the concentration levels of FA in Classrooms 1 and 2 was also calculated ($r=0.594$; $n=20$; $p<0.01$) (Table 2). The strong influence of the emission sources in Prepare room on FA concentrations in other premises of the Department was determined through a high coefficient of correlation between Prepare room and Classroom 1 ($r=0.678$; $n=20$; $p<0.01$).

Table 2. Correlations of FA concentrations in different premises of the Department

Tablica 2. Korelacije koncentracija formaldehida u različitim prostorijama Odjela

Correlations						
		FAClassroom 1	**FAClassroom 2**	**FAPrepare**	**FAStorage**	**FABreak**
FAClassroom1	Pearson Correlation	1	0.594**	0.678**	0.553*	-0.300
	Sig. (2-tailed)		0.006	0.001	0.011	0.199
	N	20	20	20	20	20
FAClassroom2	Pearson Correlation	0.594**	1	0.392	0.247	-0.374
	Sig. (2-tailed)	0.006		0.087	0.294	0.104
	N	20	20	20	20	20
FAPrepare	Pearson Correlation	0.678**	0.392	1	0.772**	-0.285
	Sig. (2-tailed)	0.001	0.087		0.000	0.223
	N	20	20	20	20	20
FAStorage	Pearson Correlation	0.553*	0.247	0.772**	1	-0.280
	Sig. (2-tailed)	0.011	0.294	0.000		0.232
	N	20	20	20	20	20
FABreak	Pearson Correlation	-0.300	-0.374	-0.285	-0.280	1
	Sig. (2-tailed)	0.199	0.104	0.223	0.232	
	N	20	20	20	20	20
**. Correlation is significant at the 0.01 level (2-tailed).						
*. Correlation is significant at the 0.05 level (2-tailed).						

The dominant influence of this emission source has been confirmed by removing the impact of FA from Classroom 2, where the correlation between Prepare room and Classroom 1 remains at a significant level ($r = 0.601$; $n = 18$; $p < 0.05$) (Table 3).

This cannot be said for the correlation between Classroom 1 and Classroom 2, which becomes moderate after removing the partial impact of FA from Prepare room ($r = 0.485$; $n = 18$; $p < 0.05$) (Table 4).

Table 3. Partial correlations of FA concentrations
Tablica 3. Parcijalne korelacije koncentracija formaldehida

Control Variables			FAClassroom1	FAClassroom2	FAPrepare
-none-[a]	FAClassroom1	Correlation	1.000	0.594	0.678
		Significance (2-tailed)	.	0.006	0.001
		df	0	18	18
	FAClassroom2	Correlation	0.594	1.000	0.392
		Significance (2-tailed)	0.006	.	0.087
		df	18	0	18
	FAPrepare	Correlation	0.678	0.392	1.000
		Significance (2-tailed)	0.001	0.087	.
		df	18	18	0
FAPrepare	FAClassroom1	Correlation	1.000	0.485	
		Significance (2-tailed)	.	0.036	
		df	0	17	
	FAClassroom2	Correlation	0.485	1.000	
		Significance (2-tailed)	0.036	.	
		df	17	0	
a. Cells contain zero-order (Pearson) correlations.					

Table 4. Partial correlations of FA concentrations
Tablica 4. Parcijalne korelacije koncentracija formaldehida

Control Variables			FAClassroom1	FAClassroom2	FAPrepare
-none-[a]	FAClassroom1	Correlation	1.000	0.594	0.678
		Significance (2-tailed)	.	0.006	0.001
		df	0	18	18
	FAClassroom2	Correlation	0.594	1.000	0.392
		Significance (2-tailed)	0.006	.	0.087
		df	18	0	18
	FAPrepare	Correlation	0.678	0.392	1.000
		Significance (2-tailed)	0.001	0.087	.
		df	18	18	0
FAPrepare	FAClassroom1	Correlation	1.000	0.485	
		Significance (2-tailed)	.	0.036	
		df	0	17	
	FAClassroom2	Correlation	0.485	1.000	
		Significance (2-tailed)	0.036	.	
		df	17	0	
a. Cells contain zero-order (Pearson) correlations.					

In order to identify the category of employees exposed to the highest concentrations of the FA and to determine the significance of the impact, additional statistical analyses were carried out. Data processing was performed using single-factor ANOVA of different groups with subsequent tests. One-factor analysis of variance determined the level of significance of the influence of FA on the health of employees in different premises of the Department.

After the conducted analysis of variance, a statistically significant difference in the concentration of FA at $p < 0.05$ was found in the measurement results at 5 locations within the Department $F(4.95) = 68.153$ $p = 0.0001$. Levene's test of the homogeneity of variance indicates the use of the corrected F value obtained by the Welch test. In addition to statistical significance, a large effect size was determined according to Cohen's criteria $\eta^2 = 0.72$.

Subsequent comparisons using the Tuckey's post hoc test indicate that the mean concentrations in the Prepare room (M= 3.45, SD= 1.68) and Storage room (M= 2.91, SD= 1.09) statistically significantly differ from the FA concentration levels in other premises. This fact points to significantly higher exposure to FA of Technical assistants compared to the other workplaces. No statistically significant differences between FA concentrations in other locations.

The levels of FA reported in this study were compared with levels reported by other researchers in anatomy laboratories. The results of this study showed that for the duration of sampling, the highest level of concentration of FA (6.60 ppm) has been detected in the Preparation room during the process of extraction of anatomical specimens from formalin as part of getting ready for the classes. This value exceeded the recommended standards established by USA-NIOSH which is 0.1 ppm, and Occupational Safety and Health Administration (USA-OSHA) which is 0.75 ppm. High levels of FA in anatomy laboratories were reported by other researchers. Akbar-Khanzadah et al. *(1994)* evaluated FA exposure in a group of 34 subjects in a gross anatomy laboratory and reported that TWA concentration of FA ranged from 0.07 to 2.94 ppm during dissecting operations. The authors reported that more than 94% of the subjects were exposed to FA in excess of the ceiling value of 0.3 ppm recommended by the ACGIH and 31.7% of the subjects exceeded the 8-h TWA action level of 0.75 ppm set by the OSHA. In addition, the authors reported irritation of eye, nose, throat, airways, and a change of forced vital capacity and forced expiratory volume in 3 s among the subjects *(Akbar-Khanzadah et al., 1994)*.

CONCLUSION

The use of formaldehyde in the preservation of human specimens has been a common practice for centuries. During the process of dissection, FA vapours are emitted from the corpses, which results in the exposure of medical students and their instructors to elevated levels of FA. The values of the FA concentrations were in range from 0.01 ppm to a maximum concentration level of 6.60 ppm.

The highest average values of formaldehyde concentration levels during the campaign were detected in the Prepare and in the Storage room with a statistically significant difference in comparison with the other sites, which distinguishes the position of technical assistant for the most vulnerable when formaldehyde exposure is observed.

The results indicate a definite necessity for the implementation of primary protection measures through improving the quality of the ventilation system, as well as the use of adequate personal protective equipment in response to the current state of air quality in the premises of the Anatomy Department.

Further research will be directed towards the promotion of a useful personal protective device, which will reduce the level of concentration of inhaled formaldehyde through effective adsorption.

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ZAŠTITA NA RADU U MEDICINSKOM LABORATORIJU I IZLOŽENOST FORMALDEHIDU

SAŽETAK: Opasne kemijske tvari u raznim oblicima narušavaju kakvoću zraka u radnoj okolini. Način unošenja otrovnih tvari u ljudsko tijelo i transformacijski putovi glavni su čimbenici štetnih učinaka. Zbog velike raširenosti formaldehida u mnogim industrijama već nekoliko desetljeća pozornost stručnjaka zaštite na radu usmjerena je na taj fenomen. Studija prikazuje temeljne karakteristike formaldehida i učinke na zdravlje prouzročene izlaganjem različitim koncentracijama te opasne tvari. Težište je na zaposlenima u medicinskim laboratorijima gdje se formaldehid koristi kao sredstvo za dezinfekciju i agens za čuvanje uzoraka tkiva u anatomskim laboratorijima. Eksperimentalni dio studije odrađen je u laboratorijima Odjela za anatomiju, a razine koncentracije formaldehida praćene su u različitim prostorijama kako bi se utvrdila izloženost zaposlenika.

Ključne riječi: *kemijske opasnosti, formaldehid, izloženost na radu, medicinski laboratorij*

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