This study was to investigate whether working in conditions of elevated concentrations of mine gases (CO₂, CO, CH₄, DMS) and dust may result in oxidative stress. Coal miners (n=94) from the Velenje Coal mine who were arranged into control group and three groups according to a number of consecutive working days. 8-iso prostane as a biological marker of oxidative stress was measured in exhaled breath condensate (EBC). Miners who worked for three consecutive days had higher 8-iso prostane values in EBC compared to the control group. Gas/dust concentrations and exposure time of a single/two day shift seem too low to trigger immediate oxidative stress.

Coal miners and miners, in general, are traditionally exposed to dust and higher concentrations of certain gases. For example, carbon dioxide (CO₂) in the inspired air in some parts of the coal mine pits reaches a concentration of 1.5%; for a short time-periods concentration raise to even more than 5%[1]. Previous research conducted in the Velenje Coal mine in 2004 found higher concentrations of several gasses, like carbon monoxide (CO), CO₂, methane (CH₄) and dimethyl sulphide (DMS)[2]. Mining process generates and disperses silicon dioxide dust into the air. In general, aluminium and iron are the main metals in the coals; therefore dust mixture usually contains particles of these substances. Such occupational/environmental exposure can induce conditions of oxidative stress development[3] and consequently lead to the increased risk of health impairment. From the literature we can see that oxidative stress can increase the risk of diseases like chronic obstructive pulmonary disease, arterial hypertension, diabetes, cancer and in general premature aging[4]. In Slovenia, for example, mining industry, among all industries, has most working days lost due to respiratory disease, gastrointestinal disease, eye disease and skin disorders[5]. Reactive species (RS) is a collective term that includes both oxygen radicals and other reactive oxygen and nitrogen species (ROS/RNS). Oxidative stress can be defined as an excessive amount of RS, which is the net result of an imbalance between production and destruction of RS (the latter is regulated by antioxidant defences). Oxidative stress is a consequence of increased generation of RS and reduced physiological activity of antioxidant defences against free radicals.

The issue of certain gases that are present in the Velenje Coal mine in high average concentrations and their potential for the formation of oxidative stress. Studies that assess the impact of methane to increase free radical formation and consequently on oxidative stress are rare.

Research Design The impact of gas and dust mixture on oxidative stress at miners was measured according to the number of working days in the mine. The control group included miners who returned to work after five days of holiday, before the start of the shift. The first group included miners who worked in the coal mine for a single day following a free weekend. The second group included miners who worked in the coal mine for two consecutive days. The third group included miners who worked in the coal mine for three consecutive days. Measurements were carried out on Monday (first group), Tuesday (second group) and Wednesday (third group), after the shift in the coal mine.

Sample Description The study involved 169 coal workers.
miners from the Velenje Coal mine. The health condition of each miner was confirmed by his general practitioner who provided written confirmation that miner’s health status is adequate. Ninety-four (n=94) coal miners passed strict inclusion criteria and were a) non-smokers, b) without chronic diseases, and c) without any drug therapy. Sample description in Table 1 shows that selected groups of miners were not significantly different in their age and number of working years in the coal mine. Informed consent was obtained from all individual participants included in the study.

**8-isoprostane as an Oxidative Stress Marker**

8-isoprostane is a prostaglandin (PG)-F2-like compound belonging to the F2 isoprostane class that is produced in vivo by the free radical-catalyzed peroxidation of arachidonic acid. It was used many times as a biomarker of lipid peroxidation and oxidative stress marker in the scientific literature. Their metabolites can be measured in urine, blood plasma, cerebrospinal fluid, exhaled breath condensate (EBC), bronchopulmonary lavage (BAL), amniotic fluid, meconium and tissues. Literature suggests that out all oxidized fatty acids, the best indicators of oxidative stress in vivo are currently the F2-isoprostanes[^6].

**8-isoprostane in EBC**

EBC was sampled with Turbo DECCS System (MEDIvAC, Parma, Italy). The sample was obtained with the single use of mouthpiece with a one-way valve and nasal clamp that prevents leakage of air through the nose. Miners were normally breathing through that mouthpiece for 10 min. Moisture in the exhaled air was then condensed into a plastic tube in a special refrigerated condenser of Turbo DECCS device. Immediately after the collection of the sample, we added 0.005% butylated hydroxytoluene stabilizer, and the sample was frozen at -40 °C. The sample was then ready for analysis. 8-isoprostane was measured with competitive enzyme immunoassay for the quantification of 8-isoprostane (8-isoprostane EIA KIT, Cayman Chemical Company, USA) by the manufacturer’s instructions. The detection limit of this immunoassay is in pg/mL range.

**Statistical Analysis**

Results were expressed as mean value ± standard deviation. Differences between multiple groups were compared using independent samples t-test and Pearson correlation coefficient for normally distributed data. In case Shapiro-Wilk test did not confirm normal distribution of data, Mann-Whitney U test and Spearman correlation coefficient were used instead. Statistical analysis was made with IBM SPSS 20.0 software (IBM Corp., Armonk, NY). Statistical significance was assumed at P<0.05.

This study was approved by the Slovenian National Ethical Committee.

In our findings, ecological monitoring of CH₄, CO₂, CO, and DMS in the coal mine are presented in Table 2. CH₄, CO₂, and CO are recorded three times

### Table 1. Sample Description, Arranged by Groups of Miners

<table>
<thead>
<tr>
<th>Items</th>
<th>Controls (n=20)</th>
<th>Group 1 (n=27)</th>
<th>Group 2 (n=26)</th>
<th>Group 3 (n=21)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>M  SD</td>
<td>M  SD</td>
<td>M  SD</td>
<td>M  SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40.3  5.2</td>
<td>39.2  5.0</td>
<td>40.5  4.5</td>
<td>39.1  5.5</td>
<td>0.642</td>
</tr>
<tr>
<td>Years at work in coal mine</td>
<td>11.8  4.3</td>
<td>12.4  5.1</td>
<td>12.6  6.5</td>
<td>10.7  4.4</td>
<td>0.496</td>
</tr>
</tbody>
</table>

**Note.** One-way analysis of variance, M: mean, SD: standard deviation.

### Table 2. Concentration of Gas in the Velenje Coal Mine Shafts and Coal Mine Courtyard

<table>
<thead>
<tr>
<th>Gas</th>
<th>Velenje Coal Mine Shafts</th>
<th>Courtyard</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>max</td>
<td>M</td>
</tr>
<tr>
<td>CH₄ (%)</td>
<td>0.18</td>
<td>0.43</td>
<td>0.26</td>
</tr>
<tr>
<td>CO₂ (%)</td>
<td>0.18</td>
<td>1.13</td>
<td>0.56</td>
</tr>
<tr>
<td>CO (ppm)</td>
<td>0.88</td>
<td>9.67</td>
<td>3.86</td>
</tr>
<tr>
<td>DMS (ppm)</td>
<td>0.49</td>
<td>57.17</td>
<td>15.16</td>
</tr>
</tbody>
</table>

**Note.** DMS is monitored in the coal mine only. *Independent samples t test, M: mean; SD: standard deviation; DMS: dimethyl sulphide.
Oxidative stress in coal miners during the shift and DMS once. Mean gas concentrations were calculated for the five-year period, data included approximately 22,000 records of CH<sub>4</sub>, CO<sub>2</sub>, CO and 7500 records of DMS from the mine excavation shafts together with 5000 records of CH<sub>4</sub>, CO<sub>2</sub>, CO from the mine courtyard. All values show increased gas exposure for miners in comparison to outdoor air.

Oxidative stress measurements resulted in a significant difference between 8-isoprostane in EBC in miners of group 3 and control group. Results in Figure 1 showed that 8-isoprostane in EBC was not increased after single day shift, the first increase was visible after two days of shift (group 2), and even more visible after three days of shift (group 3). The mean value of 8-isoprostane in EBC in control group and group 3 was 3.0±0.7 pg/mL and 5.0±1.1 pg/mL, respectively (P<0.001).

The coal miners in the Velenje Coal mine are exposed to higher concentrations of certain gases that have a potential to promote the oxidative stress. Emissions of CO<sub>2</sub>, CO, CH<sub>4</sub> and DMS in coal mine shafts were increased in comparison to values in the courtyard; yet measured values were below the threshold of Slovenian normative and were comparable to emissions measured in other coal mines of developed countries. For example, maximum CH<sub>4</sub> concentrations were always below the mining law of 1% and maximum CO<sub>2</sub> concentrations were also below the recommended threshold of 1.5%.

The major finding were significantly higher levels of 8-isoprostane in EBC of workers after being exposed to mine environment for more sequential 6-hours-shift working days. 8-isoprostane that is a member of F2-isoprostanes provides a valuable and reliable approach to assess oxidative stress in vivo.<sup>[6]</sup>

The finding that the third working day resulted in significant elevation of 8-isoprostane in EBC after the shift in comparison to the control group suggests that prolonged exposure to gas and dust mixture of three working shifts significantly influences the oxidant/antioxidant balance and thus increases the miners oxidative stress. Apart from pro-oxidant effects of inhaled gases and dust, additionally higher 8-isoprostane levels on the third day could be a consequence of depleted endogenous antioxidative defense system after prolonged exposure to moderate/low levels of oxidative stress. The antioxidative defense system is composed of the protective molecules that include antioxidant enzymes, like superoxide dismutase, glutathione peroxidases, catalase, and molecules like glutathione<sup>[7]</sup>. A second category of defense encompasses repair processes, which remove the damaged biomolecules before they accumulate to cause altered cell metabolism or viability. Antioxidant defenses can thus prevent the generation of ROS and intercept free radicals, resulting in decreased formation of 8-isoprostane<sup>[8]</sup>. Considering the later, the lower levels of 8-isoprostane after withdrawal during weekend suggest a restoration of oxidant/antioxidant balance during the two days off work.

We were unable to find previous research of 8-isoprostane measurements of coal mine workers. On the other hand studies have conducted elevated levels of 8-isoprostanes showing the occurrences of oxidative stress, for example, in asthma, COPD, acute lung injury, Alzheimer disease, liver cirrhosis and atherosclerosis patients<sup>[9]</sup>. Apart from being reliable oxidative stress markers they are also thought to have important biological effects that have a role in disease pathogenesis, their overproduction can contribute to increased platelet activation in diabetes, bronchoconstriction in asthma, vasoconstriction and decreased renal blood flow in
the hepato-renal syndrome, inhibit proteasome activity in neurodegenerative diseases\textsuperscript{[10]}.

**Study Limitations**  Our intention was to arrange four groups of 30 miners. Since we were obliged not to interfere into working process, we could only follow 47 miners after the first day of shift as 16 miners unexpectedly received other assignments and did not work their entire second consecutive day of the shift in the coal mine under increased gas and dust exposure. It was also not possible to arrange that more than 20 miners get five day holiday for a larger control group.

Since the participants were all healthy, non-smokers and the groups did closely match according to age and working years, we believe that the differences in 8-isoprostane measurements clearly represent the influence of longer exposure to mine gases and dust by consecutive working days.

We also lack the data of impact of 8-isoprostane levels in EBC to possible disease development, so we cannot recommend the upper allowed limits of 8-isoprostane in coal mine workers, what could be a useful non-invasive marker in the determination of time exposure in mine shafts. We could compare our findings to the limited extent to previously reported results of measurements of 8-isoprostane in various diseases, yet, there remains a question how important role plays oxidative stress in the pathogenesis of these diseases.

The Velenje coal mine does not measure particles of substances in the air on a regular basis therefore it is difficult to estimate if oxidative stress in miners is a consequence of increased gases concentrations or dust particles. To reduce influence of other conditions such as body fluids loss due heavy physical work we measured miners who excavated coal only and did not constructed rails for transportation or similar. In that manner typical working process of miners represented the control of heavy mining equipment.

The levels of 8-isoprostane were reported to be influenced by many factors such as PAH (polycyclic aromatic hydrocarbons) exposure from diet or passive smoking. Study participants were asked to maintain their usual food intake, usual daily activities and avoid bars on Monday and Tuesday afternoon due possibility of passive smoking and alcohol consumption. Regarding their home miners stated that family members or visitors smoke outside the premises or on the balcony.

**Recommendations for Future Research**  Correlations between urinary, plasma, and EBC of 8-isoprostane measures were significant in the first group of miners only. In that manner, it is difficult to conclude which 8-isoprostane measurement should provide most promising results. Nevertheless, as we found some significant elevations of 8-isoprostane, we suggest more testing of 8-isoprostane in EBC with similar study design. Perhaps by the extension, if allowed by a coal mine policy, for another successive day of measurements to conduct if 8-isoprostane in EBC reach elevated values in the third day and remains or extend the elevation in the fourth consecutive working day.

In conclusion, 8-isoprostane measurements in EBC seem to be a simple and reliable method for monitoring of oxidative stress in coal miners. The major finding was significantly increased 8-isoprostane levels in ECB in coal miners on the third consecutive working day, showing the increased oxidative stress after prolonged exposure. Our findings suggest that a 6-h exposure to coal mine gases and dust was a short period to cause any detectable increase of 8-isoprostane in ECB in coal miners.

**Acknowledgement**  We would like to thank all miners from the Velenje Coal mine who participated in the research.

**Conflicts of Interest**  The authors declare that they have no conflict of interest.

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**Contributions**  Study design: ZZ, MB, MMM, PK; data collection and analysis: ZZ, PK, JO, MŠ, and manuscript preparation: ZZ, MB, MMM, PK, BP, JO, MŠ.

**What this paper adds**  Our aim was to conduct how elevated concentrations of mine gases impact physiological processes in human body. Our results showed that oxidative stress in exhaled breath condensate was increasing with each consecutive day of work in the coal mine.

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