



Three Day Environmental Exposure May Trigger Oxidative Stress Development and Provoke Adaptive Response Resulting in Altered Antioxidant Activity

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Abstract

Background: We aimed to investigate the polluted working environment triggers oxidative stress and alter enzymatic antioxidant activity by a short-term interval.

Methods: The experimental study, performed in 2014, involved 94 workers from the Velenje Coalmine in Slovenia, arranged into three groups according to a number of consecutive working days in a mineshaft, supported by a control group. Levels of the antioxidant enzymes (GPx, CAT, SOD) together with TAC (the combined effect of all antioxidants) and 8-isoprostane (a biological marker of oxidative stress/damage) were measured in human plasma.

Results: Workers occupationally exposed for three consecutive working days had significantly increased 8-isoprostane biomarker, a parameter of oxidative stress ($P<0.001$). The antioxidant levels of TAC ($P<0.001$), CAT ($P<0.001$) and SOD ($P<0.001$) were all significantly decreased compared to a control group.

Conclusion: Workers in polluted working environment had significantly increased oxidative stress and altered antioxidant activity already on a third consecutive working day.

Keywords: Polluted working environment; Oxidative stress; 8-isoprostane; Enzymatic antioxidant activity

Introduction

Several studies suggest that polluted working environment involves oxidative damage (1-5). Oxidative stress can increase the risk of diseases like chronic obstructive pulmonary disease, arterial hypertension, diabetes, cancer and in general premature aging (6). Slovenian mining industry has in 2015, in comparison to other industrial fields, lost most working days because of respiratory disease, gastrointestinal disease, eye disease and skin disorders (7).

Oxidative stress is a consequence of an increased generation of free radicals, where environmental pollutants stimulate a variety of mechanisms of toxicity on molecular level as well as modulation of antioxidant enzymes (8, 9). Moreover, the lowered antioxidant level may compromise one's ability to scavenge free radicals and oxygen reactive species (ROS), which might predispose them to disease formation (10, 11). The human cell balances the potential danger of ROS by the antioxidant reserve used to oppose to the ROS dif-

fusion and to limit the propagation process. The antioxidant reserve is primarily composed of a combination of damping ROS enzymes and the non-enzymatic antioxidants (12). The first enzymatic system involved in ROS production is the superoxide dismutase (SOD). As common characteristics, all SODs accelerate thousands of times the transformation in $O_2^{\cdot-}$ and H_2O_2 . Catalase (CAT) decomposes hydrogen peroxide to water. Glutathione peroxidase (GPx) reduces lipid hydroperoxides to alcohols and hydrogen peroxide to water. This process involves also an inactivation and elimination of harmful substances to the body and it is one of the main mechanisms of protection of exposed organs (lung) or ones interested in the detoxification (liver) (13).

The 8-isoprostane is a prostaglandin (PG)-F₂-like compound and represents the major F₂ isoprostane. It is produced in vivo by the free radical-catalyzed peroxidation of arachidonic acid and its metabolites can be routinely measured in various body fluids, e.g. blood plasma, urine and exhaled breath condensate (14). The 8-isoprostane in plasma was used many times before to indicate oxidative status development in polluted working environment (15-17). Moreover, oxidative stress was studied in connection to the total antioxidant capacity (TAC) as well as SOD, CAT and GPx enzymatic activity (4, 18-20). Past studies had more or less specific study designs and conducted results were in some contradiction. For example, vitamin E and C absorption resulted in increased TAC, GPx and SOD, CAT was reduced (18). Workers also absorbed vitamins E and C, which provoked reduced TAC, SOD and CAT, GPx remained unchanged (4). Nevertheless, both proved antioxidant activity in environmentally exposed workers only, control groups remained without visible change.

We aimed to investigate whether short period of work in environment with elevated concentration of dust and gases might result in the increased oxidative stress and provoke antioxidant response.

Materials and Methods

Study design and subjects

Experimental study design was used (nonrandomized control group pretest-posttest design (21)). Data were collected, from 850 employees of the Velenje Coalmine, Slovenia, in 2014. All were invited to participate in the study and 169 male coal miners agreed to participate. The health condition of each worker was confirmed by his general practitioner who provided written confirmation that worker's health status is adequate: a) non-smoker, b) without chronic diseases, and c) without any drug therapy. Finally, 94 coal miners passed the above inclusion criteria.

This study was approved by the Slovenian National Ethical Committee. The involved coalmine workers signed written informed consent after being completely aware of the settings of the study.

The impact of polluted environment on oxidative stress of exposed workers was measured according to the number of working days in the mine (Fig. 1). The first group included workers for a single day following a free weekend (n=27). The second group included workers for two consecutive days (n=26). The third group included workers for three consecutive days (n=21). Measurements were carried out on Monday, Tuesday and Wednesday after the shift in the coalmine. Control group included workers measured before the shift after five day holiday (n=20). The control and three experimental groups were arranged according to the Velenje coal mining working schedules.

The effect of oxidative stress was measured by 8-isoprostane and enzymatic antioxidant activity by GPx, CAT, SOD and TAC (the combined effect of all antioxidants).

8-isoprostane in plasma

Blood EDTA samples were obtained from the antecubital vein. Plasma was, supplemented with 0.005% butylated hydroxytoluene and frozen at -80 °C till the analysis. The samples were then analyzed by the competitive enzyme immunoassay technique for the quantification of 8-isoprostane (8-Isoprostane EIA KIT, Cayman Chemical

Company, USA) in accordance with the manufacturer's instructions.

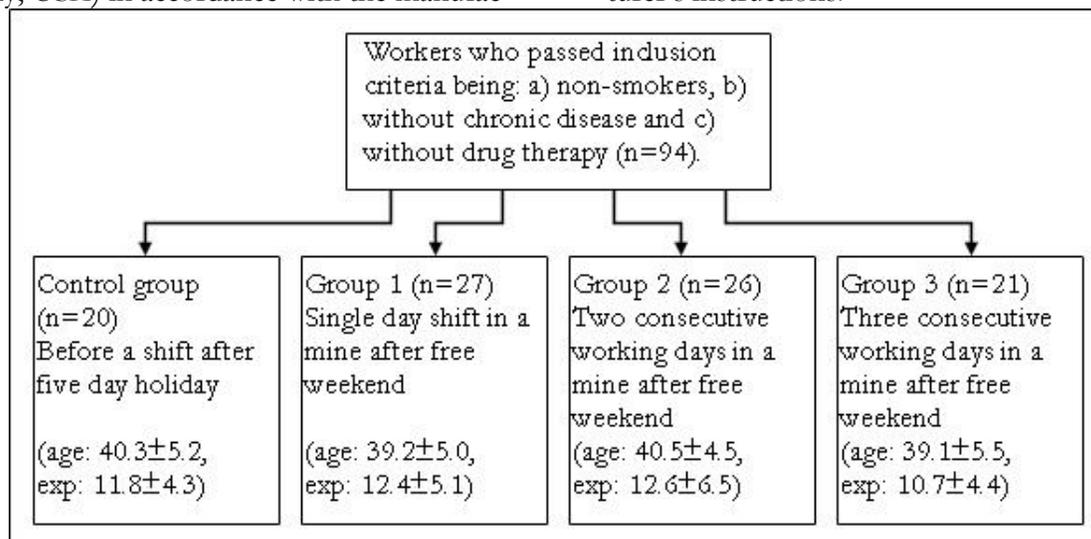


Fig. 1: Study design and sampling (age: age in years, exp: number of working years)

TAC

We determined total antioxidant status in serum with Radox TAC assay (Radox Laboratories Limited, Crumlin, UK). The assay principle is as follows: ABTS (2, 2'-azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with peroxidase (metmyoglobin) and H_2O_2 to produce radical cation $ABTS^{*+}$. This product has a relatively stable blue-green color, measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree, which is proportional to their concentration.

CAT activity assays

Catalase activity was determined by Bioxytech Catalase-520 (OXIS). The first step is hemolysis of the sample and then application on the Olympus AU 400. The procedure is done as specified in the protocol for Olympus AU 400.

GPx activity assays

For the quantitative determination of Glutathione Peroxidase (GPx) in whole blood, we used RANSEL Glutathione Peroxidase (Radox). GPx catalyses the oxidation of Glutathione (GSH) by Cumene hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH, the oxidized Glutathione (GSSG) is immediately con-

verted to the reduced form with concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance is measured at 340 nm.

SOD activity assays

The method RANSOD Superoxide dismutase (Radox) employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction INT under the conditions of the assay. The absorbance is measured at 505 nm.

Statistical analysis

Results were expressed as mean \pm standard deviation. Differences between groups were compared using independent samples t-test. The one-way analysis of variance was used to analyze groups by age and working experience. Shapiro-Wilk test has confirmed normal distribution of data. Statistical analysis was made with IBM SPSS 22.0 software (IBM Corp., Armonk, NY). Statistical significance was assumed at $P < 0.05$. All ana-

lyzed demographic characteristics and biomarkers were without missing data.

Results

Mineworkers were 39.2 ± 4.9 yr of age and had 11.6 ± 4.8 yr of working experiences in the coalmine. All workers were resident in rural areas in the surrounding of the Velenje coalmine. Selected groups of workers were not significantly different in their age (C: 40.3 ± 5.2 , G1: 39.2 ± 5.0 , G2: 40.5 ± 4.5 ; G3: 39.1 ± 5.5 , $P=0.642$) and number of working years in the coalmine (C: 11.8 ± 4.3 , G1: 12.4 ± 5.1 , G2: 12.6 ± 6.5 , 10.7 ± 4.4 , $P=0.496$). All workers graduated from a four years mining school, followed by a 14-d on-site training. After 6 months of mentorship, miners

start to work on their own. During mining career, they are obliged to attend annual education about mining safety and mining technology in order to keep their license.

Workers in group 3 occupationally exposed for three consecutive working days had significantly increased oxidative stress (8-isoprostane biomarker, $P<0.001$). Among antioxidant activity biomarkers TAC ($P<0.001$), CAT ($P<0.001$) and SOD ($P<0.001$) were all significantly decreased compared to control group (Table 1). Solely GPx remained unchanged ($P=0.744$). Figure 2 shows that oxidative stress increase resulted in antioxidant biomarkers decrease, vertical spread between the curves was evident on a third consecutive working day.

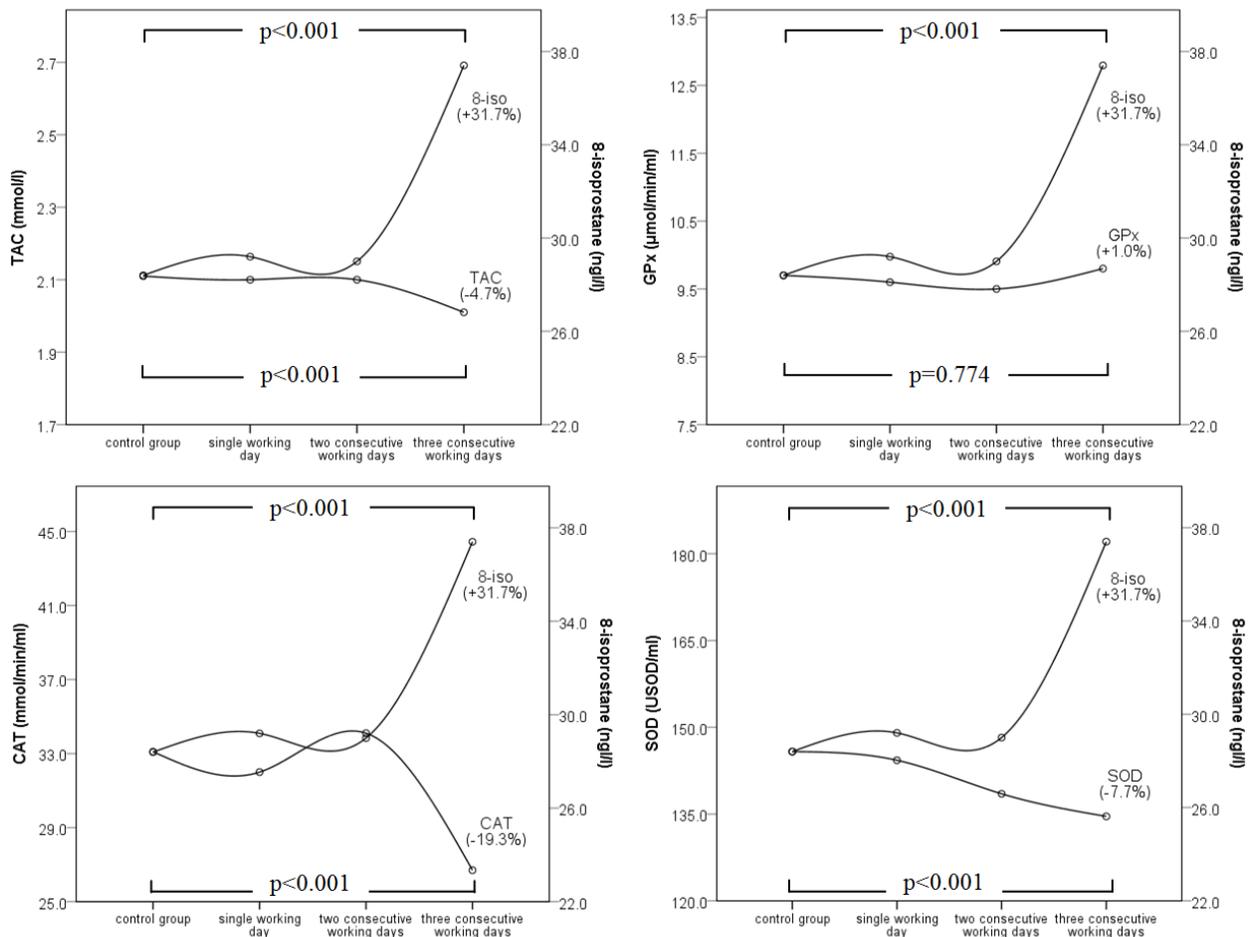


Fig. 2: TAC, GPx, CAT, SOD status and oxidative stress development in workers arranged by consecutive working days**Table 1:** Biomarkers of oxidative stress and antioxidant activity

<i>Biomarker</i>	<i>Control group (n=20)</i>	<i>Group 3 (n=21)</i>	<i>P*</i>
8-isoprostane (ng/l)	28.4±5.9	37.4±4.9	<0.001
TAC (mmol/l)	2.11±0.06	2.01±0.03	<0.001
GPx (μmol/min/ml)	9.7±2.7	9.8±1.3	0.744
CAT (mmol/min/ml)	33.1±6.9	26.7±5.9	<0.001
SOD (USOD/ml)	145.8±5.4	134.6±3.6	<0.001

* Independent samples t-test

Typical coalmine environmental pollutants are represented by coal dust mixture with CH₄ (22) and CO₂ (23) gases. Mean concentrations of CH₄ (%) and CO₂ (%) in the coal mine shafts during the three days of research were 0.25±0.07 and 0.52±0.17, respectively. Both values show increased gas exposure for workers in comparison to the outdoor air in the courtyard (0.11±0.03 and 0.08±0.03, respectively; *P*<0.001).

Discussion

Polluted working environment represents high health risk, on the other hand, physiological processes that take part in human body are less certain, especially in short term intervals. Our study concentrated on short-term antioxidant activity and did not involve an intervention to uphold antioxidant levels; workers were on normal food, for example without vitamin supplements and without any instructions for altered daily activities. Workers on a third consecutive working day had significantly elevated oxidative stress, which resulted in significantly decreased levels of antioxidant biomarkers.

Scientific research results are difficult to compare as each study has a more or less specific study design, furthermore, there is insufficient provision of interim measurements during the entire study duration and it is therefore impossible to conclude which processes took part in this time. In case of larger study time intervals authors should provide more interim results and not only

before start (first) and after the result. We tried to provide some insight on how human body in polluted working environment reacts on short term. On the third consecutive working day, 8-isoprostane increased, TAC, CAT, and SOD decreased, interpreted because of oxidative stress. Solely GPx remained unchanged. A similar picture was presented in a factory with dust mixture of lead oxides (4) and in a coalmine, yet in the latter research, GPx reacted too (18). Comparison of the results is also handicapped by the range of the reactions of enzymatic antioxidants and TAC, from a single digit percentage towards 100% or more. Yet this might be reasonable because different working environments provide different circumstances for physiological processes in a human body.

Scholar explanation of TAC proves confusing, as for example, workers in factory with dust mixture of lead oxides had higher TAC (4), on the other hand, cement plant workers had lower TAC (24). In this context, it is difficult to define the state of the initial TAC value or in other words, higher TAC being favourable or unfavourable for once health. In that manner, lower TAC is considered as increased production of free radicals (24-26) and higher TAC as a consequence of long term exposure and a result of adaptive stress responses (4, 18). Yet there seems to exist common agreement that antioxidant activity (in positive or negative direction) is influenced by environmental pollutants (1, 3, 27, 28). This phenomenon was proven by inclusion of control group, where dur-

ing the experiment no antioxidant activity was registered (4, 18). Our results showed significant antioxidant activity already on a third consecutive working day, other studies that involved experiments were very durable and lack of interim results, it is therefore unclear at what stage the antioxidant activity was neutralized. Therefore, one can argue by how long and by which frequency interventions are needed so that workers are not exposed to production of free radicals.

Redox regulation represents a very complex mechanism where it is impossible to understand fully the entire event cascade algorithm. It is clear, however, the oxidative allostasis is balanced between ROS production/reactivity and ROS protective mechanisms and the redox-responsive transcription system represents an essential part of allostasis (12). What remains unclear is at what stage/level and time particular oxidative stress-inducing compound trigger adaptive cellular responses resulting in increased antioxidant defence and repair.

This study objective was to explore how short term environmental exposure affects occupational workers. We measured 8-isoprostane as an oxidative stress biomarker in human plasma and enzymatic antioxidant activity by GPx, CAT, SOD, and TAC in coalmine workers. Our results show that workers occupationally exposed for three consecutive working days had significantly increased oxidative stress (8-isoprostane biomarker, $P<0.001$). Among antioxidant activity biomarkers TAC ($P<0.001$), CAT ($P<0.001$) and SOD ($P<0.001$) were all significantly decreased compared to control group. This may suggest that environmental exposure is also relevant on short (few days) terms, not just on longer (few weeks or months) terms as conducted in previous studies.

Limitations

Our intention was to arrange three groups of at least 30 workers. Since we were obliged not to interfere into working process, we could only follow 47 workers after first working day, as 16 workers unexpectedly received other assignments and did not spend their entire second consecutive

working day in the coalmine under increased air pollution. Anticipated control group was also reduced to 20 workers, as 10 among them had to interrupt their holidays. Since the study participants were without registered health problems, non-smokers and the groups were matched according to age and working years, the differences in antioxidant activity clearly represent the influence of longer occupational exposure to pollutants by consecutive working days.

To reduce influence of body fluids loss we measured workers who controlled heavy mining equipment for excavation of coal only. They did not installed rails for transportation or performed another sort of strenuous physical work. In addition, study participants were addressed to maintain usual food intake, avoid strenuous daily activities and social events to omit passive smoking and alcohol consumption during the three-day research period. Regarding their living area participants stated that family members or visitors smoke outside the premises or on the balcony.

Our control and experimental groups were controlled in basic demographic and health characteristics (age, number of working years, education, residence, nonsmoking status, no chronic disease, and drug prescription). Moreover, advice was given to avoid passive smoking and alcohol consumption and to perform only moderate daily activities. Certain inability to control for above-listed confounding variables (e.g. disregard of advice to avoid passive smoking, alcohol consumption and strenuous daily activities), may have influenced our results of oxidative stress development.

Sources of bias were reduced as researches could not influence sampling, all employed miners were asked to join the research by the mining company, $n=169$ agreed to participate. Part of the participants was rejected as they did not pass the inclusion criteria ($n=40$), some reported ill ($n=9$), some unexpectedly received other assignments ($n=16$), some were recalled from the holiday ($n=10$) and the rest ($n=94$) eventually took part. Researchers also could not influence on control and experimental groups formation because all the development was scheduled by the mining

company. Regarding the analysis of biological samples laboratory staff was not aware of control and experimental groups' formation. All methods used, were routine laboratory methods and were validated and performed upon manufacturer instructions. For all methods, specially designed quality control material was used. The inter- and intra- assay coefficients of variation were within the limits proposed by the manufacturer.

Since this study was composed of miners, results may not generalize to other occupational groups. In addition, concentrations of various metallic compounds in the gas/dust mixture of the breathing air may also compromise applicability and generalisability of results to other international mining areas.

Conclusion

Our sample of workers in polluted working environment had significantly increased oxidative stress and altered antioxidant activity already on a third consecutive working day. Different scientific approaches reveal more and more information on what to consider about oxidative stress to alleviate its impact on people working in polluted environments.

Ethical considerations

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interests

The authors declare that there is no conflict of interests.

References

1. Valko M, Leibfritz D, Moncol J et al (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*, 39:44–84.
2. Pelclová D, Fenclová Z, Kačer P et al (2008). Increased 8-isoprostane, a marker of oxidative stress in exhaled breath condensate in subjects with asbestos exposure. *Ind Health*, 46:484–489.
3. Auten RL, Davis JM (2009). Oxygen toxicity and reactive oxygen species: the devil is in the details. *Pediatr Res*, 66:121–127.
4. Rendón-Ramírez AL, Maldonado-Vega M, Quintanar-Escorza MA et al (2014). Effect of vitamin E and C supplementation on oxidative damage and total antioxidant capacity in lead-exposed workers. *Environ Toxicol Pharmacol*, 37:45–54.
5. Omidianidost A, Ghasemkhani M, Kakooei H et al (2016). Risk Assessment of occupational exposure to crystalline silica in small foundries in Pakdasht, Iran. *Iran J Public Health*, 45:70–75.
6. Domej W, Oettl K, Renner W (2014). Oxidative stress and free radicals in COPD-implications and relevance for treatment. *Int J Chron Obstruct Pulmon Dis*, 9:1207–1224.
7. National Institute of Public Health (2015). The lost work days statistics in Slovenia; <https://podatki.nijz.si/pxweb/sl/NIJZ%20podatkovni%20portal/>
8. Genestra M (2007). Oxyl radicals, redox-sensitive signalling cascades and antioxidants. *Cell Signal*, 19:1807–1819.
9. Halliwell B (2007). Biochemistry of oxidative stress. *Biochem Soc Trans*, 35:1147–1150.
10. Valavanidis A, Vlahogianni T, Dassenakis M, Scoullou M (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicol Environ Saf*, 64:178–189.
11. Osuji FN, Onyenekwe CC, Ifeanyichukwu M et al (2012). Antioxidant activity in HIV and malaria co-infected subjects in Anambra State,

- southeastern Nigeria. *Asian Pac J Trop Med*, 5:841–847.
12. Cornelli U, Lu C, Sun X et al (2017) *Humoral factors in the skin*. In: Practical Immunodermatology, Gao X-H, Chen H-D, eds. Springer, Dordrecht, NL, pp. 115-142.
 13. Kuipers I, Guala AS, Aesif SW et al (2011). Cigarette smoke targets glutaredoxin 1, increasing s-glutathionylation and epithelial cell death. *Am J Respir Cell Mol Biol*, 45:931-937.
 14. Janicka M, Kot-Wasik A, Kot J et al (2010). Isoprostanes-biomarkers of lipid peroxidation: their utility in evaluating oxidative stress and analysis. *Int J Mol Sci*, 11:4631–4659.
 15. Elango N, Kasi V, Vembhu B, Poornima JG (2013). Chronic exposure to emissions from photocopiers in copy shops causes oxidative stress and systematic inflammation among photocopier operators in India. *Emviron Health*, 12:78.
 16. Pelclová D, Fenclová Z, Vlčková S et al (2014). Occupational asthma follow-up--which markers are elevated in exhaled breath condensate and plasma? *Int J Occup Med Environ Health*, 27:206–215.
 17. Flaherty NL, Chandrasekaran A, del Pilar Sosa Peña M et al (2015). Comparative analysis of redox and inflammatory properties of pristine nanomaterials and commonly used semiconductor manufacturing nano-abrasives. *Toxicol Lett*, 239:205–215.
 18. Wilhelm Filho D, Avila S Jr, Possamai FP et al (2010). Antioxidant therapy attenuates oxidative stress in the blood of subjects exposed to occupational airborne contamination from coal mining extraction and incineration of hospital residues. *Ecotoxicology*, 19:1193–1200.
 19. Oliveras-López M-J, Berná G, Jurado-Ruiz E et al (2014). Consumption of extra-virgin olive oil rich in phenolic compounds has beneficial antioxidant effects in healthy human adults. *J Funct Foods*, 10:475–484.
 20. Fraile-Bermúdez AB, Kortajarena M, Zarrazquina I et al (2015). Relationship between physical activity and markers of oxidative stress in independent community-living elderly individuals. *Exp Gerontol*, 70:26–31.
 21. Dimitrov DM, Rumrill PD Jr. (2003). Pretest-posttest designs and measurement of change. *Work*, 20:159–165.
 22. Goodman GVR, Karacan CÖ, Schatzel SJ et al (2008). NIOSH research for monitoring and controlling methane at U.S. underground coal mining operations. Pittsburgh (USA): National Institute for Occupational Safety and Health, Pittsburgh Research Laboratory; <https://www.cdc.gov/niosh/mining/UserFiles/works/pdfs/nrfma.pdf>
 23. MSHA (2003). Effects of blasting on air quality. Arlington: Mine Safety and Health Administration.
 24. Pournourmohammadi S, Khazaeli P, Eslamizad S et al (2008). Study on the oxidative stress status among cement plant workers. *Hum Exp Toxicol*, 27:463–469.
 25. Suryakar AN, Katkam RV, Dhadke VN et al (2010). A study of oxidative stress in cotton industry workers from Solapur city. *Biomed Res*, 21:260–264.
 26. Permpongpaiboon T, Nagila A, Pidetcha P et al (2011). Decreased paraoxonase 1 activity and increased oxidative stress in low lead-exposed workers. *Hum Exp Toxicol*, 30:1196–1203.
 27. Elis A, Froom P, Ninio A et al (2001). Employee exposure to chromium and plasma lipid oxidation. *Int J Occup Environ Health*, 7:206–208.
 28. Poljšak B, Fink R (2014). The protective role of antioxidants in the defence against ROS/RNS-mediated environmental pollution. *Oxid Med Cell Longev*, 2014:671539.