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APPROACHES TO EXPRESS POTENTIOAL HAZARD ASSESSMENT OF NANOSIZED FRACTIONS OF WELDING FUMES

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Introduction. The impact of welding fumes (WF) remains a serious problem even in developed countries with a long history of improving the working environment. WF consists of both potentially dangerous gases and highly dispersed nanosized particles, nanosized particles, which are characterized by more pronounced biological activity and damaging effect. Screening evaluation of welding materials *in vitro* allows to obtain preliminary information on potential hazards, and is also appropriate from the standpoint of bioethics.

The aim of the study – according to the indicators of oxidative stress, as the main mechanism of damaging action of nanoparticles, to develop a method of rapid evaluation of nanoscale components.

Materials and methods of research. The damaging effect of nanosized fractions of solid components of WF (SCWF) formed during welding with high-alloy test electrodes with rutile coating was evaluated (two marks). The chemical composition of air samples was studied by inductively coupled plasma atomic emission spectrometry using an optical emission spectrometer with an inductively coupled plasma «Ortima 2100» («Perkin-Elmer», USA). Particle size was determined by dynamic light scattering using the Analysette 12 DynaSizer (Fritsch, Germany). Cattle sperm were used as a test object. Cytotoxicity was assessed using the method of rapid assessment of the toxicity of WF *in vitro.* The optical density of the obtained phospholipid extracts was determined using a ULAB 101UV spectrophotometer at a wavelength of 540 nm. Smear staining was performed according to the Lefler method (methylene blue), Main-Grunwald method (fixation) with Romanovsky staining diluted (1/3) and undiluted paint. Stained specimens were analyzed by immersion under an x1000 lens using a Charles Zeiss microscope (Germany).

Results. Nanofractions of rhutile-coated SCWF electrodes *in vitro* caused oxidative stress in the test object, resulting in morphological abnormalities, destruction of biological membranes, and release of phospholipids. The obtained data correspond to the results of *in vivo* and *in vitro* experiments, in which both studied electrodes showed cytotoxicity and damaging effect.

Conclusions. The proposed method of express assessment of WF's potential hazards significantly reduces the complexity of testing and can be used as a screening in toxicological and hygienic research at the stage of development and improvement of welding materials and/or welding technology.

Key words: welding fumes, welding electrodes, nanofractions, cytotoxicity, express assessment

Introduction

Welders without adequate protective equipment are exposed to potentially harmful welding fumes (WF). Despite considerable efforts to improve workplace safety, the impact of welding fumes remains a serious problem even in developed countries with a long history of improving the working environment [1]. For example, in Sweden, there are 71 deaths annually that can be directly attributed to WF (including coronary heart disease and lung cancer as causes of death). This is more than the total annual number of work-related deaths in all occupations in Sweden.

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There has been a downward trend in production in Ukraine over the last few decades, while unsatisfactory occupational conditions in the workplace of welders persist, and the effects of long-term exposure to harmful factors of the working environment, primarily WF, are underestimated by both doctors and employers, technologists and welders themselves [2].

The welding process is associated with a complex of unfavourable chemical and physical factors hazardous to the health of workers. It should be noted that during the professional life of a welder, the structure of production factors affecting his/her health may change many times, since working conditions may differ significantly in different branches of production, as well as in different areas of one and the same enterprise. However, the leading factor is the chemical one. Depending on the welding process, WF can contain compounds of iron, manganese, chromium, nickel, fluorine, zinc, aluminium, silicon, cadmium, lead, as well as nitrogen and carbon oxides, ozone etc. It is known that WF are composed of both potentially hazardous gases and highly dispersed nanoparticles.

It has been established that the leading fraction of the solid component of WF (SCWF) is represented by particles in the nanoscale range [1, 3]. Nanoscale particles have a more distinct biological activity and damaging effect: due to their size, they can penetrate the skin, enter the bloodstream, as well as directly the brain via nerve endings [4]. Consequently, nanosized fractions of SCWF are hazardous, with their toxicity depending on the composition of the welding material and the reactivity of the particles, which leads to the need for toxicological and hygienic studies, whose results should be considered when developing new materials/technologies and protective strategies.

At the same time, it should be noted that European and American specialists point out that modern alternative methods for testing substances of chemical and biological origin should in many cases replace the conventional toxicological experiments on laboratory animals. In this regard, it should be noted that the toxicity of nanoparticles is primarily due to the development of oxidative stress, peroxidation of membranes, followed by an increase in their permeability, dysfunction and destruction. Screening evaluation of new materials (including welding materials) by *in vitro* methods provides preliminary information about the potential danger and is also reasonable from the bioethical point of view. Therefore, the development of scientific approaches to rapid assessment of hazard of nanoscale objects of different chemical nature and composition is one of the priority tasks of modern preventive medicine, which will contribute to clarification of mechanisms of damaging effects on the body of nanoscale components, improvement of assessment of their hazardous effects and protection.

The aim of the study is to develop a method of rapid evaluation of nanoscale WF fractions by oxidative stress indicators as the main mechanism of damaging effect of nanoscale components.

Materials and methods of research

The damaging effect of nanosized fractions of solid components of SCWF formed by welding with high-alloy rutile type electrodes («14–32») with low content of chromium (VI) (chromium (VI) was not found in hygienic assessment) has been assessed, average aerodynamic diameter of SCWF particles was 101.72 nm; as well as with serial electrode Crystal with rutile type of coating with mass fraction of chromium (VI) 0.9 %, average

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aerodynamic diameter of SCWF particles – 148,5 nm. Other main components of investigated electrodes «14–32» and «Crystal» are chromium (III) (3.91 % and 0.71 % respectively), nickel (mass fraction of 1.39 % and 0.74 % respectively) and manganese (mass fraction of 5.2 % and 10.33 % respectively).

Nanoscale fractions of SCWF were sampled according to the method for determination of nanoparticles in workplace air [5]. The chemical composition of air samples was studied by inductively coupled plasma atomic emission spectrometry using an Ortima 2100 optical emission spectrometer with an inductively coupled plasma (Perkin-Elmer, USA). Particle size was determined by dynamic light scattering using the Analysette 12 DynaSizer (Fritsch, Germany). Cattle sperm were used as a test object. The potential hazard of SCWF nanofractions was carried out using the method of rapid assessment of the damaging effect of nanomaterials based on the content of membrane lipids of bull spermatozoa *in vitro* [6]. The optical density of obtained phospholipid extracts was determined using ULAB 101UV spectrophotometer at 540 nm. Cytotoxicity was evaluated by the method of express evaluation of WF toxicity *in vitro* [7].

For morphological studies of spermatozoa after exposure (1 h, t = 37 °C) to nanosized fractions of SCWF formed after welding with test electrodes, frozen ejaculate smears were prepared together with the test biomaterial by evenly distributing a drop of bio-liquid on the subject. Part of the preparations was air dried and the other was fixed with ethanol. Smears were stained using the Lefler technique (methylene blue), Maine-Grunwald (fixation) with Romanowsky staining with diluted (1/3) and undiluted dye. Stained products were analyzed by immersion under x1000 objective using a Carl Zeiss microscope (Germany).

Results of the research and their discussion

It was found that nanosized manganese (1.1 μg/m^3), zinc (0.051 μg/m^3) and silicium (0.06 μg/m^3) were detected in the workplace air after welding with the pilot electrode «14–32». In turn, nanosized chromium (VI) (1.0 μg/m^3), zinc (0.02 μg/m^3) and silicium (0.05 μg/m^3) were detected in samples taken after welding with a serial electrode «Crystal».

It should be noted that in the body lipid peroxidation reactions substrate are lipophilic compounds localized in membranes and other lipid structures, and initiators are hydrophilic reactive oxygen species, intermediate lipophilic radical peroxidation products may participate in further development of the process [8]. Consequently, the phospholipid composition determines the liquid-crystalline properties and permeability of the membrane. Membranes ensure the normal function of protein transporters, enzymes that catalyse oxidation processes, cellular respiration and oxidative phosphorylation. The intensification of lipid peroxidation processes depends on the degree of damage to membranes of biological objects. In turn, the choice of bovine spermatozoa as a test-object is also caused by the fact that despite their relatively short life span, their biological features (plasma membrane and acrosome, which are lipoproteid and glycoproteid units, the density of protein and nucleus packing, low water content, low level of metabolism in the immobile state) result in greater resistance to external influences.

On the other hand, spermatozoa are more sensitive to oxidative stress than other cells, due to their small cytoplasm volume, low concentration of antioxidants and DNA repair systems, and large amount of polyunsaturated fatty acids, which are easily subject to peroxidation. In addition, the structure of spermatozoa is such that antioxidant

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enzymes are unable to protect the cell membrane at the tail and acrosome level. The germ cell response to stress is the disruption of a number of important biochemical processes, including the functioning of membrane structures, and the activation of a suicide program – apoptosis. Apoptosis is characterized by a whole set of stage-specific biochemical changes occurring in both the nucleus and the cell membrane of the sperm cell, among which, for example, changes in phospholipid dynamics. The accumulation of lipid peroxides in tissues is accompanied by the destruction of the molecular structure of the membranes. It should also be taken into account that it is bovine spermatozoa used in express method of determining cytotoxicity of WF *in vitro*, which requires the use of a serial «Image Analyzer AT-05» (RF) [7].

The potential hazard of nanofractions of SCWF was evaluated using a method for rapid assessment of the damaging effect of nanomaterials by the content of bovine sperm membrane lipids *in vitro* [8]. The method involves exposure of bovine gametes to nanomaterials in glucose-citrate buffer, followed by washing with chilled trichloroacetic acid (2.5 %), extraction of phospholipids from the sediment with a chloroform-methanol (2:1) mixture, and determination of phospholipids in the obtained extract (the amount of which depends on the degree of cell membrane damage).

Determination of the optical density of the obtained phospholipid extracts at 540 nm indicated the destruction of the molecular structure of sperm membranes exposed to nanofractions of SCWF and the release of phospholipids (Table 1).

As can be seen from Table 1, the optical density of SCWF sample of Crystal electrode was 1.023 times higher than that of SCWF sample of electrode «14–32». These data correspond to the results obtained by the standard express method of cytotoxicity determination *in vitro*, in which the serial electrode «Crystal» showed slightly worse values than the experimental electrode «14–32» (toxicity index (47.81 ± 4.70) % and (64.03 ± 2.20): approximately 1.3 times higher cytotoxicity) (Table 2). As the toxicity index (It) is equal to the ratio of the mobility parameters of the indicator cell suspension in the experimental sample to the

Table 1

Optical density and transmittance of experimental and control phospholipid extracts

Sample	Wavelength, λ, nm	Transmittance, T, %	Optical density, A
Control extract	540	98.6 ± 0.8	0.0085 ± 0.0010
Experimental extract of nanofractions of SCWF «Crystal»	540	26.8 ± 0.14	0.571 ± 0.001
Experimental extract of nanofractions of SCWF «14–32»	540	27.7 ± 0.1	0.558 ± 0.002

Table 2

Cytotoxicity of experimental brands of electrodes

Sample	Experimental brand of electrodes	Coating of electrodes	Toxicity index, %
1	14–32	Rutile	64.03 ± 2.20
2	Crystal	Rutile	47.81 ± 4.70

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mobility parameters of the indicator cells in the control sample. At the value of It in the range of 70–120 % the experimental solution is considered non-toxic.

The data obtained in the rapid lipid peroxidation test were confirmed by morphological analysis of abnormal spermatozoa exposed to welding materials. There was an increase in the number of abnormal cells and a decrease in the proportion of morphologically normal cells compared to controls (the proportion of abnormal sperm cells in controls was 22 %, in samples exposed to TCA electrode «14–32» – 72 % and electrode «Crystal» – 86 %). Attention was also drawn to the presence of residual corpuscles both in the case of the electrode «14–32» and the electrode «Crystal», which probably resulted from the death of sperm cells due to peroxide damage to the membranes and their morphological anomalies (Figure 1).

The revealed germ cell defects under the action of the investigated nanosized fraction of CSWF developed in different sections. In particular, among the defects of spermatozoa most often head and tail abnormalities were revealed. Thus, in the head they were characterized by changes in its shape (pear-shaped and triangular), inclination, frequent absence of the acrosomal area or reduction of its size, and edema processes. Defects of the tail were characterized by its shortening, looping of different sizes and spiralling in all sections.

A characteristic feature of the sperm tail is motility, where the terminal part of the apparatus of movement represents the most important part. Disturbance therein inhibits motility or leads to its complete absence (asthenozoospermia). In particular, in the experiment, abnormal twisting of the terminal region into a loop was observed in both specimens studied. Special attention should be paid to the simultaneous detection of double and triple anomalies in different sections of the same spermatozoa (Figure 2). This complex of morphological abnormalities of spermatozoa reflects reduced motility and functional fullness. In addition to certain sperm anomalies, their aggregation (clumping) was observed, indicating their pathological state, accompanied by swelling of the head, thinning of the tail etc.

The data of preliminary studies on the biological effects of nanofractions of SCWF electrodes *in vivo*, in particular, of histological studies month after a single intratracheal administration to male Wistar rats (0.5 ml of FCSA nanofractions solution, 10 animals in each group) showed that in rats exposed to «14–32» SCWF nanofractions the histological

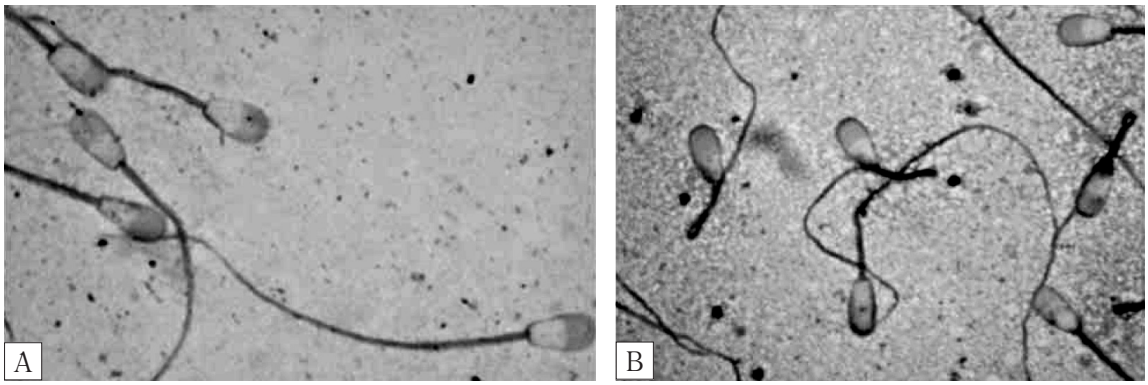

Figure 1. Bovine spermatozoa:

A – control, B – exposed by nanofractions of SCWF serial electrode «Crystal» (the presence of a significant number of residual cells)

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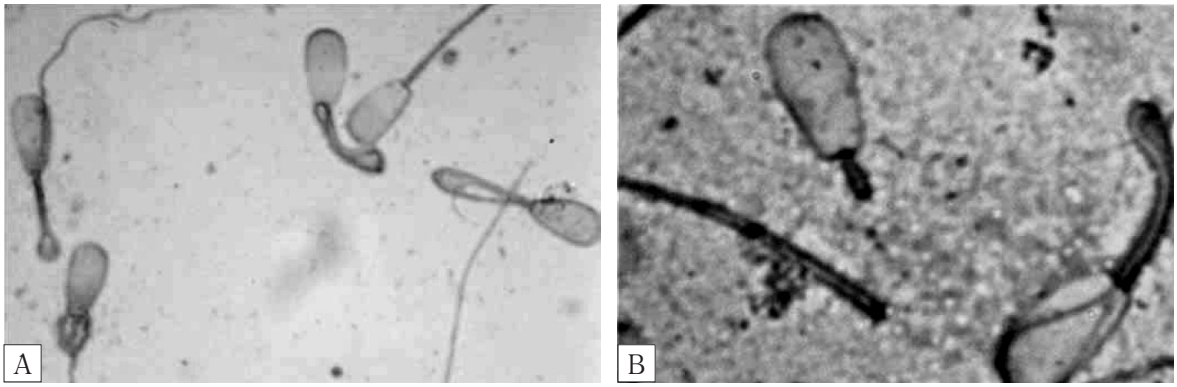

Figure 2. Defects of spermatozoa:
A – triple defects in spermatozoa exposed with SCWF nanofractions of the electrode «Crystal» (head – swelling, tilt, reduction of acrosomal area; middle part – swelling; tail – twisting into a middle section loop); B – triple defects exposed by electrode «14–32» nanofractions (head – swelling, no acrosome; middle part – thickening; tail – shortening and swelling in all sections)

structure of lungs did not differ significantly from that of control, while lung tissue of rats exposed to SCWF nanofractions containing chromium (VI) showed marked venous ulceration of the organ [9]. Histological examination of the liver of the rats, exposed to chromium (VI)-containing SCWF nanofractions revealed complete preservation of the trabecular structure characteristic for the organ, while the results of histological examination of the liver of the rats, exposed to SCWF nanofractions of electrode «14–32» revealed dystrophic changes in hepatocytes, having mainly diffuse character. At the same time, marked hyperplasia and hypertrophy of stellate macrophagocytes with the signs of their marked dystrophy were revealed in the organ under the influence of nanofractions of SCWF «14–32», which probably developed in cells due to the increased functional activity, which may be conditioned by the influence of nanosize mangan.

Conclusions

1. It was found that nanofractions of rutile-coated SCWF electrodes in *in vitro* experiments induce oxidative stress in the test object, which leads to destruction of biological membranes and phospholipid release.
2. The exposure of the test-object to SCWF nanofractions formed by commercial welding electrode «Crystal» and by experimental electrode «14–32» with improved hygienic properties and containing no chromium (VI) caused morphological abnormalities in germ cells.
3. The data obtained by method of express assessment of determining the damaging effect of SCWF nanofractions by the membrane phospholipid content and confirmed by morphological examination of the test object correlate with the results of *in vivo* and *in vitro* experiments in which both the tested electrodes showed cytotoxic and damaging effects.
4. The proposed method of express assessment of WF potential hazards significantly reduces the complexity of testing and can be used as a screening in toxicological and hygienic research at the stage of development and improvement of welding materials and/or welding technology.

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