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NEUROLOGICAL AND NEUROPHYSIOLOGICAL EXAMINATIONS OF WORKERS EXPOSED TO ARSENIC LEVELS EXCEEDING HYGIENE STANDARDS

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Abstract
Objectives: The assessment of the neurotoxic effect of arsenic (As) and its inorganic compounds is still the subject of interest due to a growing As application in a large array of technologies and the need to constantly verify the principles of prevention and technological parameters. The aim of this study was to determine the status of the nervous system (NS) in workers exposed to As at concentrations exceeding hygiene standards (Threshold Limit Values (TLV) – 10 μg/m^3, Biological Exposure Index (BEI) – 35 μg/l) and to analyze the relationship between the NS functional state, species of As in urine and As levels in the workplace air. **Material and Methods:** The study group comprised 21 men (mean age: 47.43±7.59) employed in a copper smelting factory (mean duration of employment: 22.29±11.09). The control group comprised 16 men, matched by age and work shifts. Arsenic levels in the workplace air (As-A) ranged from 0.7 to 92.3 μg/m^3; (M = 25.18±28.83). The concentration of total arsenic in urine (As^{tot}-U) ranged from 17.35 to 434.68 μg/l (M = 86.82±86.6). **Results:** Syndrome of peripheral nervous system (PNS) was manifested by extremity fatigue (28.6%), extremity pain (33.3%) and paresthesia in the lower extremities (33.3%), as well as by neuropathy-type mini-symptoms (23.8%). Electroneurographic (ENeG) tests of peroneal nerves showed significantly decreased response amplitude with normal values of motor conduction velocity (MCV). Stimulation of sural nerves revealed a significantly slowed sensory conduction velocity (SCV) and decreased sensory potential amplitude. Neurophysiological parameters and the results of biological and environmental monitoring showed a relationship between As^{tot}, As^{III} (trivalent arsenic), the sum of iAs (As^{III}+As^{V} (pentavalent arsenic))+MMA (monomethylarsonic acid) concentration in urine and As levels in the air. **Conclusions:** The results of the study demonstrate that occupational exposure to inorganic arsenic levels exceeding hygiene standards (TLV, BEI) generates disorders typical of peripheral neuropathy.

Key words:
Occupational exposure, Arsenic speciation, Nervous system, Neuropathy, Neurophysiological test

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INTRODUCTION

Adverse effects of arsenic (As) and its inorganic compounds have been known for a long time. Pathologies in the cardiovascular, respiratory, digestive, central and peripheral nervous systems, as well as the damage to the optic, auditory and olfactory nerves have been reported [1–3]. Of these systems, the peripheral nervous system (PNS) is the most critical target. Arsenic neuropathy is characterized by sensory disorders, which are more frequent and more serious than motor impairments [4].

In occupational exposure, As effects are usually combined with carcinogenicity [3]. The reports on toxic effects of inorganic As on nervous system (NS) concern mostly workers involved in the process of copper ore refining in nonferrous metal industries. Clinical and neurophysiological studies in the copper-mill workers were carried out by Blom et al. [5] and Lagerkvist and Zetterland [6].

The study groups were exposed to ambient air levels of arsenic changing over time. The clinical studies have not revealed the presence of severe forms of neuropathies described earlier by other authors [7]. Electroneurographic (ENeG) tests showed the slowing down of both motor and sensory peripheral nerve conduction velocity (NCV). Based on long-term observations, various authors emphasize that the PNS function is evidently influenced by the duration of exposure to As and its compounds [6].

Difficulties in assessing the nervous system status arise from the problems encountered in estimating exposure levels and possible combined exposure to arsenic and other metals, such as lead (Pb), copper (Cu) and zinc (Zn) occurring in copper smelting factories [8,9].

Hygiene standards for individual harmful agents have been developed, however, the combined effect of several metals and their interaction has not as yet been elucidated. It is well known from the literature review that simultaneous exposure to copper and zinc are antagonists to neurological effects of lead [9,10].

New assessment principles, based on measurements of individual As chemical forms in urine, used in biological monitoring, allow for the more precise assessment of occupational exposure [11,12]. It is recommended that the determination of the sum of iAs (iAs = $As^{III}+As^{V}$), MMA and dimethylarsinic acid (DMA) be involved in the biological monitoring of arsenic exposure.

The aim of this study was to determine the status of the nervous system, with special attention paid to the PNS, in workers exposed to arsenic and its inorganic compounds at concentrations exceeding the hygiene standards and to analyze the relationship between the NS functional state and the total urine excretion of arsenic (As^{tot}-U), species of arsenic forms, As^{III}-U, As^{V}-U, the sum of iAs ($As^{III}+As^{V}$)+DMA+MMA, the sum of iAs (($As^{III}+As^{V}$)+MMA) as well as arsenic levels in the workplace air.

MATERIAL AND METHODS

Study population

Neurological and neurophysiological examinations were performed on the outpatient basis. The study group comprised 21 men (aged 35–59 years; mean (M) ± standard deviation (SD) = 47.43±7.59) employed in a copper smelting factory and exposed to arsenic and its inorganic compounds (Table 1).

Of the 61 workers employed in ten copper smelting factories, workers showing highest concentrations of As^{tot} in urine were eligible for the study. The workers were employed as refiners of non-ferrous metals, copper electrolyzers and crane operators. The employment duration ranged from 1 to 37 years (M±SD = 22.29±11.09) (Table 2). Workers aged over 50 (42.85%) (Table 1) and employed for 21–30 years (33.3%) predominated in the study group (Table 2).

The workers suspected of the present or past neurological diseases, especially craniocerebral traumas, neuroinfections, cerebrovascular diseases, migraines, radicular

Table 1. Study and control groups by age

Group	Respondents							
	total (n)	age (years)				age group [n (%)]		
		min.	max	M	SD	≤ 39 years	40–49 years	≥ 50 years
Study	21	35	59	47.43	7.59	4 (19.04)	8 (38.09)	9 (42.85)
Control	16	27	57	46.06	9.57	4 (25.00)	4 (25.00)	8 (50.00)
Fisher test	n.s.	–	–	–	–	–	–	–

min. – minimal value; max – maximal value; M – mean; SD – standard deviation; n.s. – non-significant.

Table 2. Study group by exposure duration

Exposure duration (years)				Exposure duration in study group (N = 21) [n (%)]				Total [n (%)]
min.	max	M	SD	≤ 10 years	11–20 years	21–30 years	≥ 31 years	
1	37	22.29	11.09	4 (19.1)	5 (23.8)	7 (33.3)	5 (23.8)	21 (100)

Abbreviations as in Table 1.

syndrome and ophthalmopaties were excluded from the study.

The workers with diabetes, hypertension and those suspected of alcoholism, smoking and abuse of medicines were also excluded. The values of arsenic levels in the air (As-A) and the results obtained from the biological monitoring of total arsenic in urine (As^{tot}-U), As^{III}, As^{V}, indicators representing the sum of iAs ($As^{III}+As^{V}$)+DMA+MMA and iAs ($As^{III}+As^{V}$)+MMA excreted with urine, were chosen for assessing the results of clinical and neurophysiological examinations.

The control group comprised 16 men, aged 27–57 years (M±SD = 46.06±9.57). The control group was matched by age and work shifts. Workers of the control group were employed as carpenters, fitters and wiremen in repair workshops, as well as forklift truck drivers in the same plant.

Exposure assessment

The values of arsenic levels in the air (As-A) and the results obtained from the biological monitoring of total arsenic in urine (As^{tot}-U), As^{III}, As^{V}, the indicators being the sum of iAs ($As^{III}+As^{V}$)+DMA+MMA and iAs ($As^{III}+As^{V}$)+MMA excreted in urine, were chosen for assessing the results of neurophysiological examinations.

Air samples were collected via individual dosimetry method in workers' breathing zones. Urine and blood samples were collected after the shift. Before samples collection workers were asked to remove their work clothes and wash hands. The workers were asked about the consumption of fish meals and smoking. This study was approved by Ethics Committees of Nofer Institute of Occupational Medicine in Łódź, Poland (decision No. 3/2012).

Analysis of As levels in the air (As-A)

Air samples were collected via individual dosimetry in the workers' breathing zones, continuously throughout 6–7 h, in accordance with the sample collecting strategy described in PN-Z-04008-7 [12]. During the collection process. individual dust collecting devices (Personal

Air Samplers, Vortex Standard and Vortex Standard 2 by Casella, and also EHA AIR-300 by Ekohigiena) were used. The samples were collected on membrane filters made of cellulose nitrate (Sartorius 11304) or fiberglass (Whatman GF/A). Inductively coupled mass spectrometry (ELAN DRC-e ICP-MS) with Dynamic Reaction Cell (Perkin Elmer, SCIEX, USA), were used for arsenic determination.

Analysis of As concentration in urine (As-U)

Urine samples were frozen after collection and stored at < –20°C until analysis. Prior to dilution, urine samples were centrifuged at 4000 rpm for 10 min, then the supernatant was diluted tenfold by 1% HNO_3 for total arsenic and mobile phase for speciation analysis.

ELAN DRC-e ICP-MS with Dynamic Reaction Cell (Perkin Elmer, SCIEX, USA) was used for arsenic determination. The instrument Series 200 HPLC (Perkin Elmer, SCIEX, USA) was used to separate arsenic from chemical forms.

Methods of neurological and neurophysiological examinations

The process of neurological diagnosis comprised subjective (anamnesis) and physical examinations. The anamnesis provided information about the major neurological complaints and symptoms potentially associated with exposure to inorganic arsenic. The condition of cranial nerves, motor and sensory systems, and coordination were assessed during the physical examination. The resultant data were recorded on a neurological examination chart – the same for all the study participants.

Electroneurographic tests were performed using a Neuromatic 2000 C unit (Dantec, Denmark). The medial nerves in the upper extremities and the peroneal nerves in the lower extremities were stimulated in each subject to assess peripheral nerve motor fibers. The sensory fibers were stimulated in the sural nerves of the lower extremities. The tests were carried out in accordance with a commonly accepted research methodology [13].

The conduction velocity of the surveyed nerves, distal motor latency, response amplitude and amplitude of nerve sensory potentials were assessed. Stimulation was performed using surface electrodes, types 13K60 and 13L36 (Dantec, Denmark).

Visual evoked potentials (VEPs) were recorded using a Neuromatic 2000 C (Dantec, Denmark). We used monocular full field checkerboard stimulation, and the frequency of pattern reversal was 2 Hz.

Receiving electrodes were placed as follows: active electrode at Oz, 3 cm above the straight line, and reference electrode at Fz. The latency of components N1 P100 and N2 and the difference in P100 latency in the right and left eye stimulation, amplitudes N1 P100 and P100 N2 were assessed. A total of 2×200 individual responses were averaged.

Statistical analysis

In the statistical analysis of the results, the Fisher F test, Fisher exact test, and Pearson's correlation coefficients were employed. All statistical analysis was performed using Statistica StatSoft® Polska software package.

RESULTS

Assessment of the levels of arsenic in air and arsenic species in biological material

Arsenic levels in the workplace air ranged from 0.7 to 92.3 μg/m^3 (M±SD = 25.18±28.83) (Table 3). In the study group, the TLV (10 μg/m^3) was exceeded in 12 (57.14%) workers.

Concentrations of As^{tot}-U ranged from 17.35 μg/l to 434.68 μg/l (M±SD = 86.82±86.6) (Table 3). In the exposed group, the values of biological exposure indices (BEI) for urine As concentrations were exceeded in 18 (85.72%) workers (standard BEI for urine As = 35 μg/l).

Table 3. Assessment of occupational concentrations of inorganic arsenic (iAs) and its species in urine (U) and levels of arsenic in the workplace air

Parameter	Study group (N = 21)			
	min.	max	M	SD
Urine concentrations of total arsenic and its species (μg/l)				
As^{tot}-U	17.4	434.7	86.82	86.60
As^{III}-U	0.3	22.8	9.93	7.41
As^{V}-U	0.7	14.5	5.38	3.87
Sum iAs (As^{III}+As^{V})+MMA	3.4	51.1	22.38	14.22
Sum iAs(As^{III}+As^{V})+DMA+MMA	15.2	108.6	56.23	29.54
Air levels of arsenic in the workplace (μg/m^3)	0.7	92.3	25.18	28.83

As^{tot} – arsenic total; As^{III} – trivalent arsenic; As^{V} – pentavalent arsenic; iAs – inorganic arsenic; MMA – monomethylarsonic acid; DMA – dimethylarsinic acid.
Other abbreviations as in Table 1.

Blood Pb (Pb-B) concentration ranged from 71 μg/l to 468 μg/l (M±SD = 254.05±118.73). In none of the cases the value of adopted BEI for lead (500 μg/l) was exceeded. Serum Cu (Cu-S) concentrations ranged from 0.80 to 1.82 mg/l (M±SD = 1.13±0.22, reference values: 0.8–1.6 mg/l). Serum Zn (Zn-S) concentrations ranged from 0.59 to 0.97 mg/l (M±SD = 0.76±0.12, reference values: 0.8–1.4 mg/l).

Neurological and neurophysiological examinations

The incidence of neurological symptoms is presented in Table 4.

The reported central nervous system (CNS) complaints were mainly manifested by headaches (38.1%), increased emotional irritability (47.6%), sleep disorders, such as insomnia (33.3%) or excessive somnolence (38.1%). Headaches reported by the patients were usually of mild to moderate severity and varying in location without other symptoms, such as nausea, vomiting, pulsation, photophobia. They occurred and intensified towards the end of working time. The state of the increased emotional irritability was manifested by irritation, anxiety and fatigue. They generated conflicts at work and in the family life.

Difficulty in falling asleep and sleep maintenance, frequent or early waking up (33.3%) and excessive somnolence during the day (33.1%), reported by those exposed, were found to be the other significant sleep problems. The respondents did not link these symptoms with work shifts. Neurological symptoms, such as dysmnesia (23.8%), problems with concentration (19%) and mood lability (19%) were less frequently recorded. The frequency of CNS subjective manifestations was higher in the study than in the control group. The frequency of reported emotional irritability was significantly higher in the exposed subjects.

Neurological examinations did not reveal the presence of focal symptoms of organic CNS lesions.

Peripheral nervous system symptoms were manifested by myospasms in and fatigue of the lower extremities (23.8 and 28.6%, respectively). The respondents did not associate these symptoms with their posture at work. Extremity pain (33.3%) and paresthesia (33.3%), characterized by formication, were mostly located in distal segments of the lower extremities. The incidence of extremity pain, paresthesia and fatigue of the lower extremities was significantly higher in the exposed workers. On objective neurological examination, PNS lesions in the lower

Table 4. Incidence of neurological symptoms in the study and control groups

Symptom	Study group (N = 21) [n (%)]	Control group (N = 16) [n (%)]	Probability values calculated with the Fisher's exact probability test
Headache	8 (38.1)	2 (12.5)	0.082
Vertigo	2 (9.5)	0	0.204
Increased emotional irritability	10 (47.6)	2 (12.5)	**0.024**
Dysmnesia	5 (23.8)	1 (6.3)	0.151
Concentration difficulty	4 (19.0)	0	0.065
Insomnia	7 (33.3)	3 (18.8)	0.322
Excessive sleepiness	8 (38.1)	2 (12.5)	0.082
Mood lability	4 (19.0)	0	0.065
Anxiety and fear	1 (4.8)	0	0.376
Spasms in extremity muscles	5 (23.8)	2 (12.5)	0.384
Muscular fatigue	6 (28.6)	0	**0.019**
Extremity pains	7 (33.3)	0	**0.010**
Paresthesia	7 (33.3)	0	**0.010**
Objective disorders of peripheral nervous system	5 (23.8)	0	0.036

The values in bold represent statistically significant values, $p < 0.05$.

extremities were revealed in 5 (23.8%) workers. The decreased Achilles reflexes with marked asymmetry and peripheral sensory neuropathy in the lower extremities were found in 3 and 2 cases, respectively. The incidence of these symptoms was significantly higher in the exposed group of workers.

Based on the statistical analysis with use of Pearson's correlation coefficient, the relationship between the nervous system and the duration of exposure (years) was assayed (Table 5). A significant relationship was found between the duration of exposure and CNS symptoms, such as vertigo, problems with concentration, insomnia, excessive sleepiness and mood lability, as well as between duration of exposure and myospasm, pain in the lower extremities and paresthesia.

The results presented in Table 6 concern assessment of the absence or presence of NS symptoms (0/1) and determination of relationship between the level of species of As in urine and As concentration in the workplace air.

Table 5. Relationship between neurological symptoms in the study group (N = 21) and exposure duration (years)

Symptom	Pearson's correlation coefficient (r)
Headache	0.052
Vertigo	**0.426**
Irritability	–0.219
Dysmnesia	0.316
Concentration difficulty	**0.514**
Insomnia	**0.439**
Excessive sleepiness	**0.632**
Mood lability	**0.435**
Anxiety and fear	0.263
Spasms in extremity muscles	**0.522**
Muscular fatigue	0.237
Extremity pains	**0.635**
Paresthesia	**0.635**
Disorders of peripheral nervous system	0.274

The values in bold represent a considerable strength of relationship (over 0.4).

Table 6. Irregularities on the part of the nervous system [0/1] in the study group in relation to arsenic (As) species levels in urine and arsenic concentration in air (Fisher F test)

Symptom	Arsenic species in urine of As-exposed workers (N = 21) (μg/l)									As-A ($\mu g/m^3$)
	As^{tot}-U	As^{III}-U	As^{V}-U	DMA	MMA	AsB	Sum iAs+ DMA+ MMA+ AsB	Sum iAs+ DMA+ MMA	Sum iAs+ MMA	
Headache										
Vertigo				++						
Irritability			+							
Dysmnesia										
Concentration difficulty						+				
Insomnia										+
Excessive sleepiness										
Mood lability	+					+	+			
Anxiety and fear	+++					+++	+++			
Spasms in extremity muscles										
Muscular fatigue	+				++		+	+		
Extremity pains										
Paresthesia	+			+			+	+		
Disorders of peripheral nervous system			+							

The values statistically significant, + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$.
AsB – arsenobetaine.
Other abbreviations as in Table 3.

The results of electroneurographic tests are summarized in Tables 7 and 8.

The stimulation of the medial nerve motor fibers showed a significant decrease in response amplitudes as compared with the control group, as well as a significant difference in the value of standardized distal latency (Table 7).

More significant electrophysiological alterations were found during stimulation of the peroneal nerves. A bilateral slowing down of motor conduction velocity (MCV), significant after the right nerve stimulation and a significant bilateral decrease in response amplitudes were shown (Table 7). The value of standardized distal latency was significantly prolonged after stimulation of the right peroneal nerve.

Lesions in the sural nerve were characterized by significant slowing down of sensory conduction velocity (SCV) and significant decrease in the amplitude of nerve sensory potential (Table 7).

The statistical analysis with use of Pearson's correlation coefficient showed significant relationship between the value of total arsenic in urine and MCV in the right

Table 7. Electroneurographic (ENeG) findings in the study and control groups

Group	Motor fiber stimulation								Sensory fiber stimulation		
	medial nerve right			peroneal nerves					sural nerve right		
				right			left				
	conduction velocity (m/s)	response amplitude (mV)	standardized distal latency (ms/cm)	conduction velocity (m/s)	response amplitude (mV)	standardized distal latency (ms/cm)	conduction velocity (m/s)	response amplitude (mV)	standardized distal latency (ms/cm)	conduction velocity (m/s)	sensory potential amplitude (μV)
Study (N = 21)											
M	55.27	7.56	0.51	48.85	6.45	0.64	50.00	6.02	0.62	47.12	8.47
SD	7.10	3.97	0.05	5.44	1.96	0.08	7.05	1.73	0.06	6.57	2.55
Control (N = 16)											
M	58.16	17.86	0.59	54.32	16.81	0.58	53.53	17.49	0.59	60.03	17.26
SD	7.67	2.57	0.06	4.85	2.95	0.05	4.76	3.09	0.07	5.65	5.37
Fisher F test	0.245	**0.001**	**0.001**	**0.003**	**0.001**	**0.029**	0.094	**0.001**	0.258	**0.001**	**0.001**

The values in bold represent statistically significant values, $p < 0.05$.
Abbreviations as in Table 1.

peroneal nerve (Table 8). Rather strong (> 0.3), albeit insignificant relationship between these parameters after the stimulation of the left nerve was evidenced. Moreover, rather strong (> 0.3) relationships between the sum of iAs+DMA+MMA, the sum of iAs+MMA and the standardized value of the distal latency after stimulation of the right peroneal nerve were observed.

The analysis of the relationship between As levels in the workplace air and ENeG parameters demonstrated a considerable association with the value of standardized distal latency after stimulation of the right peroneal nerve and statistically significant relationship with the response amplitude after the left nerve stimulation (Table 8).

No statistically significant relationship was evidenced between the values of As chemical forms in urine, As levels in the workplace air and the analyzed ENeG parameters after stimulation of the medial nerve motor fibers.

The values of Pearson's correlation coefficients, presented in Table 8, showed rather strong (> 0.3), but insignificant relationship between air As levels and bilateral SCV in the sural nerves.

The results of visual evoked potentials, presented in Table 9, revealed a bilateral prolongation of the major positive component P100, significant as compared with the control group ($p < 0.05$). The difference in latency from the stimulation of the right and left eyes did not exceed the adopted value of 6–7 ms. The comparison of the P100 amplitude between the results obtained in both groups, study and control, showed lower values in the exposed subjects. A statistically significant difference was found in amplitude P100 N2 after stimulation of the right eye.

Based on the statistical analysis with use of Pearson's correlation coefficient (Table 10), rather strong relationship between As^{III}-U concentration and amplitudes N1 P100 and P100 N2, as well as the relationship between the sum of iAs+MMA and the latency of the N1 component after the left eye stimulation were revealed. A considerably strong relationship between As levels in the workplace air

Table 8. Relationship (r-values) between motor (MCV) and sensory conduction velocity (SCV) in peripheral nerves in the study group (N = 21) and the concentrations of arsenic (As) chemical forms in urine and air levels of arsenic (based on Pearson's correlation coefficient)

Parameter	Urine levels of individual chemical forms of arsenic (µg/l)					Air levels of arsenic in the workplace (µg/m^3)
	As^{tot}-U	As^{III}-U	As^{V}-U	Sum iAs (As^{III}+As^{V})+ DMA+MMA	Sum iAs (As^{III}+As^{V})+ MMA	
Medial nerve, right						
conduction velocity	–0.017	–0.134	–0.264	–0.047	–0.258	–0.156
response amplitude	–0.058	–0.237	0.019	–0.076	–0.130	–0.078
standardized distal latency	–0.162	–0.020	0.046	–0.100	–0.019	0.068
Peroneal nerve, right						
conduction velocity	**–0.422***	–0.201	0.008	–0.113	–0.163	0.022
response amplitude	–0.034	0.154	–0.216	–0.179	–0.007	–0.105
standardized distal latency	–0.224	–0.284	–0.286	**–0.397**	**–0.354**	**–0.305**
Peroneal nerve, left						
conduction velocity	**–0.378**	–0.242	–0.097	–0.057	–0.213	–0.022
response amplitude	–0.207	–0.316	0.012	–0.152	–0.191	**–0.532***
standardized distal latency	–0.033	–0.086	–0.057	–0.067	–0.024	–0.200
Sural nerve, right						
conduction velocity	0.164	0.076	–0.232	–0.109	–0.062	**0.406**
sensory nerve potential amplitude	–0.112	0.074	0.026	0.021	0.114	–0.124
Sural nerve, left						
conduction velocity	0.146	0.189	0.044	–0.025	0.110	**0.425**
sensory nerve potential amplitude	–0.102	0.068	0.082	0.044	0.124	–0.161

The values in bold represent a considerable strength of relationship (over 0.3).
* $p < 0.05$.
Abbreviations as in Table 3.

and the latency of N1 and P100 components after the left eye stimulation was noted. As regards VEPs amplitude, a significant relationship was evidenced between As levels in the ambient air and the value of the P100 N2 amplitude after the right eye stimulation, as well as a substantially strong association was noted between As level in the air and the N1 P100 and P100 N2 amplitudes after left eye stimulation.

Table 9. Latency and amplitude of visual evoked potentials (VEPs) in the study and control groups

Group	VEP latency (ms)						VEP amplitude (μV)			
	stimulation of the right eye			stimulation of the left eye			stimulation of the right eye		stimulation of the left eye	
	N1	P100	N2	N1	P100	N2	N1 P100	P100 N2	N1 P100	P100 N2
Study (N = 21)										
M	82.48	104.95	129.52	82.24	103.90	127.05	5.82	5.48	6.10	6.34
SD	14.32	12.88	16.74	10.16	11.23	12.89	2.31	2.69	2.69	3.39
Control (N = 16)										
M	71.13	97.25	122.44	68.00	96.25	125.06	6.99	7.23	6.78	7.69
SD	4.19	5.41	12.37	5.27	5.75	10.38	3.18	2.67	2.88	2.94
Fisher F test	**0.004**	**0.031**	0.164	**0.001**	**0.018**	0.618	0.206	**0.050**	0.468	0.212

The values in bold represent statistically significant values, $p < 0.05$.
N1, P100, N2 – latency; N1 P100, P100 N2 – amplitude.
Other abbreviations as in Table 1.

Table 10. Relationships (r-values) between visual evoked potentials (VEPs) in the study group (N = 21) and the concentration of arsenic (As) chemical forms in urine and air levels of arsenic in the workplace (based on Pearson's correlation coefficients)

VEP parameter	Eye	Urine levels of individual chemical forms of arsenic (μg/l)					Air levels of arsenic in the workplace ($\mu g/m^3$)
		As^{tot}-U	As^{III}-U	As^{V}-U	Sum iAs ($As^{III}+As^{V}$)+ DMA+MMA	Sum iAs ($As^{III}+As^{V}$)+ MMA	
Latency N1	RE	0.154	0.026	–0.262	0.131	–0.035	–0.257
Latency P100	RE	–0.042	0.084	–0.097	0.113	0.007	–0.113
Latency N2	RE	–0.122	–0.126	–0.013	0.056	–0.157	–0.252
Latency N1	LE	0.049	–0.299	–0.240	–0.039	**–0.308**	**–0.331**
Latency P100	LE	–0.079	–0.090	0.007	0.118	–0.090	**–0.318**
Latency N2	LE	0.025	0.114	–0.045	0.162	0.035	–0.159
Amplitude N1 P100	RE	–0.159	0.179	0.046	–0.101	0.027	0.269
Amplitude P100 N2	RE	–0.069	0.185	–0.017	–0.018	0.053	**0.552****
Amplitude N1 P100	LE	0.071	**0.394**	–0.060	0.027	0.180	**0.322**
Amplitude P100 N2	LE	0.251	**0.416**	–0.042	0.171	0.290	**0.418**

RE – the right eye; LE – the left eye.
The values in bold represent a considerable strength of relationship (over 0.3).
** $p < 0.01$.
Other abbreviations as in Table 3.

DISCUSSION

Clinical disorders of the neuropathic nature in the nervous system were observed in workers employed in the copper smelter factory and exposed to As inorganic compounds in conditions of exceeded hygiene standards.

The subjects of the study group did not show focal lesions of organic damage to the central nervous system. However, the exposed workers reported CNS complaints, but there were no grounds for diagnosing them with toxic encephalopathy. On physical neurological examination, minor lesions (e.g., PNS mini-symptoms) were observed in the exposed workers. Symptoms, such as myospasm, pain in the lower extremities and paresthesia were significantly related with the duration of exposure to As and its inorganic compounds. Lagerkvist and Zetterland emphasized the evident effect of exposure duration on PNS functions [6]. Reported complaints and so called mini-symptoms with concurrent abnormalities in the ENeG test may indicate subclinical neuropathy or early phase of this syndrome [14]. Frequently, these complains need to be confirmed by the additional diagnostic value of sural nerve biopsy [15].

Of all the neurophysiological examinations performed under this study, ENeG test revealed the most visible lesions. They were mainly manifested by significantly lower response amplitude after stimulation of motor fibers of the medial and peroneal nerves, compared with the control group. The MCV values in these 2 nerves were within the standard laboratory limits [16].

The results of our own studies are in agreement with the observation made by Murphy et al. [17] and Seppäläinen [18] who emphasized an axonal nature of arsenic neuropathy. They analyzed the course of arsenic neuropathy and concluded that axonal lesions predominated in the early stage, whereas demyelinated changes prevailed in the later stage as secondary to the neurogenic process.

Our own studies have not revealed any relationship between ENeG parameters of the medial nerve and the concentration of arsenic and its chemical species in urine and As levels in the workplace air.

Araki et al. (1993) were inclined to believe that disorders of conduction velocity in peripheral nerves of the upper extremities (radial and medial) indicated lead toxicity [10]. The stimulation test of the sural nerve showed in the study group a significant slowing of sensory conduction velocity and the decreased amplitude of sensory potential compared with the control group. Tseng et al. [19] express the opinion that slowing down of sensory conduction velocity and changes in sensory potential amplitude of the sural nerves are the markers of early As neuropathy.

The presented results of electrophysiological tests showed substantially strong relationships between ENeG parameters of the peroneal nerves and As^{tot} in urine, the sum of iAs+DMA+MMA, the sum of iAs+MMA in urine and As level in the workplace air. A substantially strong relationship was also revealed between bilateral sensory conduction velocity in the sural nerves and As level in the workplace air.

The VEPs test was performed in view of the earlier reports on the cases of retrobulbar damage to the optic nerve resulting from As exposure [1].

Our tests showed significant alterations in the latency of the major component P100 compared with the control group and insignificant decrease in the potential amplitude. They also revealed considerably strong relationship, especially between VEP amplitude and the value of As^{III} in urine and As level in the workplace air. The results suggest the presence of optic neuron disorders in the course of As exposure.

In the presented study the confounding effect of Pb on the nervous system was not analyzed. In the study group, blood Pb concentration (254.05 μg/l) was well below the BEI (500 μg/l). Neither occupational lead poisoning nor disturbances in heme synthesis were diagnosed in the subjects under study. Disturbed VEP test result observed

in our study seems to result from arsenic exposure. Exposure to Pb even at higher levels did not produce disturbance in VEP test in our study. Langauer-Lewowicka and Kazibutowska (1991) who assessed the usefulness of the evoked potential method in surveying people exposed to inorganic lead under conditions of exceeded Polish hygiene standards for Pb, have not revealed any departure from VEP parameters [20].

It is rather difficult to give a clear answer to what extent the observed clinical and neurophysiological disorders can be associated with neurotoxic effect of As or combined (As+Pb) exposure. Some findings suggest a possible synergetic effect of exposure to arsenic and lead [21].

The analysis of occupational exposure of workers in the copper smelter factory, the nature and localization of clinical and neurophysiological lesions, the characteristic feature of the optic neuron dysfunction and the correlations between biological monitoring parameters and inorganic arsenic levels in the workplace air strongly support the hypothesis of the predominating neurotoxic effect of inorganic arsenic. Compared to the studies reported earlier [22,23], results presented in this paper were obtained with the use of ICP-MS and apply to a different population of workers exposed to inorganic arsenic.

Monitoring of the effects of occupational exposure to arsenic and its inorganic compounds on the nervous system, as well as the outcomes of determining urine concentrations of As species should contribute to the prevention of As exposure and provide the direction for further studies.

CONCLUSIONS

1. Based on the results of the neurological studies, it has been evidenced that occupational exposure to arsenic and its inorganic compounds in copper smelting factories at concentrations exceeding hygiene standards generates neuropathic disorders in the peripheral nervous system.
2. Association was observed between neurophysiological (ENeG and VEPs) parameters and As levels in the workplace air, in single cases with the values of As^{tot}-U, As^{III}-U and the sum of iAs+MMA concentrations in urine.
3. Electroneurographic tests along with the assessment of typical parameters, including motor and sensory conduction velocity, amplitude and latency, should be made obligatory, especially in workers with symptoms of neuropathy. For screening assessment useful in medical practice, the examination of the peroneal and sural nerves should be strongly recommended. The scope of neurolophysiological examinations should be expanded, depending on individual indications. The tests of visual evoked potentials may provide valuable information on the functional state of the optic nerve.

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