Registered cases of occupational n-hexane intoxication in Bucharest

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Abstract: The article presents the characteristics of 7 cases of chronic occupational n-hexane poisoning admitted in the Bucharest Clinic of Occupational Diseases in 2012-2013 from the clinical, biomarkers and evolution perspective in comparison with data from the scientific literature. The objective of the analysis was to identify the main characteristics of these cases.

Results: All cases presented with peripheral and central nervous system symptoms. Delayed etiological diagnosis, long evolution and slow rehabilitation were the most important clinical findings. Transitory increase of inflammatory markers might be the expression of the oxidative mechanisms involved in the neurotoxic effect.

Conclusions: The number of registered occupational n-hexane intoxication cases is low compared to other countries, most probably due to the lack of law compliance regarding toxic exposure declared by the employer, occupational medicine surveillance and toxicological control. Poor management of the occupational risk was the main cause of intoxication in these patients.

Key Words: n-hexane, occupational exposure, neurotoxic effects, inflammatory markers.

N-hexane is a straight-chain, 6 carbons alkane obtained from fractional distillation of petroleum mixtures. Despite being classified as toxic for humans, it is estimated that European Union manufacturies use 10000 - 100000 tones/year [1].

N-hexane is a component of different solvents, glues, paint thinners, degreasers. Workers are frequently exposed to a mixture of organic solvents exposure. N-hexane is used in chemical industry, in petroleum extraction and distilleries, leather and shoemaker manufactures, furniture, electronic, plastic and rubber industry, and at workplaces such as: printing of laminate products, spray drawing, labs and vegetal oil extraction. Only some processes of the food industry are allowed to use n-hexane: fat fractioning, preparation of low fat products. It is forbidden to use this solvent in the cosmetic industry.

Although it is used at such a large scale, except the cases we have declared, no other n-hexane occupational intoxication has been declared in Romania during 2012-2013. It is one of the reasons we consider this presentation of interest for the medical community. All subjects representing our cases worked in small leather and shoe manufacturies. Six of the cases represent a group of collective poisoning, while the seventh represents a singular case, with severe and prolonged symptomatology. The patient’s co-workers had minor symptoms.

The physic and chemical characteristics of n-hexane are important for its hazardous effects: it is a volatile liquid at room temperature, reaching rapidly high concentration in the inspired air of confined, unventilated spaces. The vapours are colourless and cannot be visually detected. Persons exposed to n-hexane

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experience an initial euphoria state ("the well-being effect"), characteristic for the majority of the organic solvents and will avoid using personal protection equipment, if not specifically educated about the risk. The working environments of our patients were small workshops, without any ventilation system in place, except for natural ventilation. The patients were admitted during winter, when work was done with closed windows. This seasonal particularity of the occupational n-hexane intoxication has been previously reported [2]. Even more, the workers were not informed about the exposure, never been instructed on the risk management procedures and no individual protection measures were provided. All these factors contributed to the chronic intoxication. Therefore, we can consider that poor risk management and lack of legal compliance is the common cause of these diseases.

Another characteristic of this substance is its relatively high lipid diffusion: which explains why n-hexane enters the body through skin, accumulates in the fat tissue, in brain and peripheral nervous system but also in liver, spleen, kidneys and suprarenal glands.

The most important route of exposure remains the airways route: accounting for up to 80-90% of the total body content [2, 3]. The pulmonary uptake is approximately 20% of the total concentration in the inspired air [4]. This relatively small proportion is due to the fact that a large amount of the inhaled n-hexane is eliminated during expiration (does not pass the alveolar membrane) and that another part is detoxified at the pulmonary level to hexanoic acid. In our patients, the route of exposure was both respiratory and dermal. The boxes of glue containing n-hexane were opened directly on the working tables and vapours reached the respiratory airways easily. None of the patients wore masks or gloves. As previously mentioned, mechanical ventilation was absent.

In the liver, n-hexane is detoxified via transformation to 1-hexanol, 1-hexanal and hexanoic acid. The hexanoic acid enters the fatty acids beta-oxidation cycle of the fatty acids [3]. There is also another metabolic pathway in the liver, that leads to the toxic metabolite, 2.5-hexanediol (2.5 HD). 2.5 HD is generated on the monooxygenase pathway, depending on CYP2E1 of P450 cytochrome [5]. The genetic variability of this enzyme determines the level of activity of this metabolic pathway: previous studies showed that the homozygote genotype CC of CYP2E1 Dra involves a significantly higher risk of peripheral neuropathy after n-hexane exposure than homozygotes with the DD (CC) genotype (adjusted OR=5.58, 95% CI=1.32–23.65) [6]. Besides CYP2E1, specific reactivity might be the consequence of other genes polymorphisms, such as GLUT1 and GLUT2 involved in detoxification or in the ones related to neuronal growth (such as the neuronal vascular factor, angiogenin, described for the diabetic neuropathy) [7]. We don’t have such genetic characteristics for our patients, but this might be one of the explanations why only one patient working in the same working conditions as other 30 employees had a severe neurologic disorder.

2.5 HD is water-soluble and it is easily distributed inside the body. Hepatic detoxification via glucuronidation and urinary elimination of 2.5-hexanediol - free and conjugated form - are the main metabolic pathways in humans. In intoxications, the liver threshold of glucuronidation is overpassed and the concentration of free 2.5 HD in urine increases. 2.5 HD is considered a relevant exposure indicator, reflecting the quantity of n-hexane that has been absorbed inside the body. 2.5 HD is also the final product of metabolism of methyl n-butyl ketone. This explains the synergic effect of exposure to both substances and is important for the exposure assessment. In our cases, the occupational history didn’t identified methyl n-butyl ketone in the workplace environment of our patients. All patients had high urinary levels of 2.5 HD, even though it was measured 2 weeks after exposure cessation.

In exposed subjects, 2.5-HD increases the activity of soluble guanylyl cyclase (sGC) induced by nitric oxide (NO) in the lymphocytes of the peripheral blood [8]. The level of urine 2.5-HD was not correlated with the degree sGC activation in lymphocytes from the exposed subjects. This raises questions about the efficiency of the periodic determination of the 2.5-HD; a reasonable alternative would be replacing or adding biological effect indicators to the monitoring process, but we don’t have such a cost-effective screening test available.

The current recommendations for the periodical medical control remain the clinical examination and the urinary measurement of 2.5-HD. Our patients had no pre-employment medical examination, no periodical medical examination, no annual urinary 2.5-HD measurement, as stipulated by the law. These elements contributed to such diagnostic delay (more than 3 months from the onset) of the neuropathy in all cases.

2.5 HD is a neuronal toxic. After acute exposure, the central nervous symptoms that predominate are headache, euphoria, dizziness, hallucinations, and narcosis that can lead to respiratory arrest. The severity of the symptoms is correlated with the environmental concentration. The chronic exposure leads to both peripheral and central nervous system effects. The mixt peripheral neuropathy is one of the most characteristic clinical manifestation of the disease. The initial complaints are symmetric, sensitive symptoms (numbness, burning sensation, light touch, paraesthesia); these symptoms are located in the extremities of the limbs. The motor component of the neuropathy is expressed by distal muscular weakness, diminished or even abolished deep tendon reflexes and muscular atrophy. The common element of our cases was symmetric mixed peripheral
neuropathy (Table 1). On a detailed and careful history, we found the onset to be dominated by the sensitive component. These symptoms were ignored by the patients as they were linked to fatigue. The neurological symptoms were due to unprotected exposure (the lack of ventilation system, manual application of the glue without gloves). For the peripheral neuropathy, the repetitive movements and the non-ergonomic sitting position were other contributing factors. Two weeks after exposure cessation, one of the cases presented with both increased levels urinary hippuric acid and 2.5-HD, that documents concomitant exposure to other organic solvents.

The peripheral neuronal effect is suggested on electromyography (EMG) by low conduction velocity, focal conduction block, dispersion of the muscular component of the action potential and longer distal latency [2, 9, 10]. The anatomic lesion is axonal degeneration and focal demyelination ("dying back neuropathy"). In our cases, all patients presented EMG criteria of demyelination neuropathy and one patient also presented, a bilateral carpal tunnel syndrome.

Chronic central nervous system exposure leads to an organic affective syndrome: sleep disorders, irritability, psychic functions impairment, hypersensitivity to light or noise and depression [11]. All our patients reported physical asthenia and 2 of them lost weight due to diminished appetite.

If the exposure continues, patients might experience personality disorders, and even severe encephalopathy with dementia. This latest form is due to the structural lesions of the brain; clinic signs are represented by depression, inability to perform usual simple tasks, memory impairment, and modification of the verbal fluency. On electroencephalography the alpha rhythm organisation is affected, slow waves are diffusely increased, the beta wave frequency increases or spikes and wave complexes might suggest a lowering threshold of neuronal excitability [12, 13]. One of our patients had auditory evoked potentials that were linked to her ENT medical history of otitis. In experimental models, n-hexane impairs the auditory function. Current experts’ opinion is to consider n-hexane as a potential ototoxic agent [14].

Like other organic solvents, 2.5-HD induces an axonopathy with giant paranodal swelling and myelin retraction, with clustered mitochondria and neurofilaments into the peripheral zone. The evolution leads to axonal degeneration. The mechanism implies the reaction between 2.5-HD and the lysine amino acids of the protein structures with primary pyrrole adducts formation and autoxidation. Pyrrolic dimers are formed and these dimers become responsible for protein crosslinking of the neurofilaments [15].

The common mechanism of action of the organic solvents is oxidative stress [16]. In sub-acute intoxications, the level of free oxygen radicals increases and this affects the membrane permeability [17] and extracellular leakage of lactate dehydrogenase [18]. The effect is well expressed in neuronal ends but also in the CNS, with inhibition of the neurogenesis, and impairment of the microglia function (increased expression of the microglia marker Mac 1). In vitro, inhibition of the neural differentiation is correlated with the level of ROS induced by the 2.5HD. Proteomic studies showed a reduction in the expression for proteins involved in maintaining the cytoskeletal integrity, the redox potential and in the protein folding process associated with an increased of expression of the enzymes involved in the energetic metabolism: pyruvate kinase, creatine kinase, malate dehydrogenase [19]. Chronic experimental exposure to 2.5-HD (in cell cultures, in animal models) modifies the glutamate–nitric oxide–cyclic GMP, diminishing the activation of sGC by nitric oxide (NO). In normal conditions, NO bounds to the hem group of the sGC raising up to 400 times its enzymatic activity [8]. The sGC transforms GTP in 3,5 cGMP, which acts as second messenger in synaptic transmission, being involved in learning and memorization, and the reduction of vascular resistance in brain vessels. Impairment of this pathway is associated with endothelial dysfunction and inflammation and is correlated with increased reactive protein C levels. From this perspective, we consider important to underline the fact that all our patients had, at admission, some positive inflammatory markers (reactive protein C, ESR). This is an observation that hasn’t been communicated until now. A larger study is needed to validate this association. The inflammatory markers returned to normal values without any specific anti-inflammatory treatment after exposure cessation. Our observation is in accordance with same experimental data in mice, which showed increased metal protein activity and acute phase proteins, such as fibrinogen, after n-hexane exposure [20].

Besides oxidative stress, reduced level of dopamine and homovanillic acid in corpus striatum, the cytotoxic effect on the neuron cytoskeleton proteins and on the ubiquitin positive protein conjugates determines CNS dysfunction. These CNS effects are indirect arguments on a possible role of n-hexane in Parkinson disease [21] as this has been described for other types of
solvent exposure.

Recovery from peripheral neuropathy implies a period of spasticity and hyperreflexia. After exposure cessation, rehabilitation is slow, (up to 1-3 years) even for medium severity cases; for the severe forms, symptoms may last for several years [9, 13]. All patients were evaluated 1 year after the onset. Our patients had a slow recovery, according to the degree of severity of the neuropathy: in mild cases, patients recovered in less than 1 year, the very severe ones didn’t recuperate in 2 years and still have significant sequelae: distal atrophy, sensorial deficit, muscular weakness, diminished achillean reflex. Rehabilitation was slower, particularly when the motor component was well expressed.

CONCLUSIONS

1. The occupational intoxication with n-hexane is rarely and tardily diagnosed in Romania, compared to other European countries [22] most likely because of the sporadic toxicological control of exposure indicators (2,5-HD). The indicators of biological effect are used only for diagnostic purposes (EMG, audiogram). Patients with long term exposure have high urinary 2,5-HD. This high value was maintained for months after exposure cessation in our cases. Without a targeted occupational history and the determination of exposure indicators, the diagnosis is very difficult and therefore the etiological diagnosis is ignored.

2. From the time of diagnosis, the evolution is long and includes both peripheral and CNS symptoms, with initial aggravation. Rehabilitation is slow, particularly for the motor component of the neuropathy.

3. Oxidative mechanisms and acute phase reactants have an important role in generating the neurotoxic effect. All our patients had transitory increases of plasma inflammatory markers. These markers normalized after exposure cessation without any anti-inflammatory medication.

4. Lack of periodical medical control and the absence of collective and individual protection measures (that are legal obligations for all workplaces containing toxic hazards) were the major risk factors for the intoxication. Poor management of the occupational risk was the main cause of disease in these patients. Individual susceptibility cannot be excluded in some cases, but it doesn’t substitute or exclude the accountability of the employer for the poor risk management. The reduction at the minimum possible level of n-hexane in the workplace environment by technical measures, the education of the employees on risk management procedures and the provision of occupational medicine services to the employees are legal obligations of the employer and should be strictly implemented.

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