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Toxic effect of polymetalic industrial dust on white mice during ecotoxicological experiment. I. Changes in cell morphology and proliferation

S. Petkova, M. Topashka-Ancheva**, Y. Martinova*, P. Vasileva*

**Institute of Experimental Morphology and Anthropology, Bulgarian Academy of Sciences, Sofia*
***Institute of Zoology, Bulgarian Academy of Sciences, Sofia*

The effect of industrial polymetallic dust on white mice during ecotoxicological experiment has been studied. Industrial dust with known composition, mixed with balanced animal food in concentration of 1% was given to experimental animals during 150 days. All mice were injected with Tritiated thymidine. Pieces of liver, kidney, spleen, testes and bone marrow were proceeded for routine morphological and autoradiographic study. Inflammation and lipid degeneration of hepatocytes and massive destruction of kidney cortex and medula was observed. Pyknosis of megakariocyte nuclei and supression of cell proliferation-proliferation was registered as well. These results have shown that the industrial polymetallic dust exerts a toxic effect on morphology of key organs of hematopoietic, digestive and secretory systems of white mice.

Key words: industrial dust, white mice, cell morphology, autoradiography.

Introduction

Technical revolution and technologies resulted an enormous environmental pollution lately. The life organisms including humans are exposed on this noxa through nutrition chain, breathing and drinking water. Biological barrier is limited and very specific to different pollution and not very effective. This resulted accumulation of toxic elements followed by harm effect on the whole organism. One of the biggest source of environmental pollution in Bulgaria is Metallurgic Plant "Kremikovtsi", emitting a polymetallic industrial dust as a waste product, rich in Mn, Fe, Al, Mg, Ca, Si etc. All this elements actively take part in the metabolism but the higher concentrations in the environment are toxic to the organisms. High doses of Al in drinking water cause neurodegenerative damage, demency, encephalopathy, Alzheimer's disease etc [4]. In addition AlCl3 introduced per os in rats provokes pathologic changes in the spleen and lymph nodes, expressed as a follicle hyperplasy and germinal centers enlargement [2]. Morphological changes in liver and kidney as well as alterations in protein and carbohydrate metabolism are described [1]. The concentration of Fe is important since excess or deficit acts negatively on the organism. An increase in lung cancer incidence and tuberculosis

is registered between metallurgists, miners and founders [5]. Industrial dust rich in Fe results functional disorders in kidney, adrenal gland and on the hormonal balance. Intoxication with Mg decreases sharply blood pressure and causes kidney disfunction [3]. As a result of chronic exposure to Mn neuropsychic disorders, Parkinson disease and liver cirrhosis are described [7]. Increased concentrations of Si and Ca in the environment induce functional changes in the respiratory, digestive and immune systems. Pathological changes in the genome (chromosomal aberrations) has been described as well [6, 10].

In our previous study we have monitored the effect of polimetallic industrial dust as a waste product of "KCM-Plovdiv", rich in Pb, Cd. We have established a cytotoxic and genotoxic effect of the waste on hematological parameters and on the thymocyte ultrastructure [8, 9].

Except from fieldwork, it is possible to obtain as well in laboratory an efficient information about the impact of toxicants. The specific reaction observed in laboratory mice help to clarify the effects of pollution. The aim of the present study was to expand these studies on the effect of industrial polymetallic dust as a waste product of MP "Kremikovtsi" on white mice during an ecotoxicological experiment.

Materials and Methods

The study was conducted over a 150-day period from June-October 2000 year. 60 white mice (males) ICR strain, were divided into a control group of 25 mice and an experimental group of 35 mice. Control mice (mean weight – 28.9g) and experimental mice (mean weight - 27.2g) were housed in a vivarium with temperature 20°C, humidity 45-75% and photoperiod 7 AM – 7 PM.

For the experimental treatment a conventional balanced animal food mixed with polymetal industrial dust have been given the animals in small glass containers. Polymetal dust, obtained from the waste products of the "Kremikovtsi" plant, was mixed in 1% concentration: 50g dust with 4.950kg animal food.

Control animals have received only balanced animal food. All mice were allowed access to food and water *ad libitum*.

The composition of the polymetal dust (mg/kg) was determined by atomic emission spectrometry (AES) with inductively coupled plasma (ICP): phosphorus (P) – 4940mg/kg; potassium (K) – 6769mg/kg; magnesium (Mg) – 1844mg/kg; calcium (Ca) – 1073mg/kg; sulphur (S) – 1371mg/kg; iron (Fe) – 178mg/kg; manganese (Mn) – 102mg/kg; aluminium (Al) – 34mg/kg; silicon (Si) – 98mg/kg; lead (Pb) – 77mg/kg; zinc (Zn) – 46mg/kg; copper (Cu) – 7.5mg/kg; nickel (Ni) – 1.3mg/kg; cadmium (Cd) – 0.35mg/kg; vanadium (V) – 1.25; arsenic (As) - <0.2mg/kg; cobalt (Co) - <0.1mg/kg; selenium (Se) - <0.4mg/kg.

Samples from control and experimental mice were taken on day 15-, 40-, 60-, 90-, 120- and 150.

Cyclophosphamide (CP)(70mg/kg) was used as a positive control.

The animals were injected with 3H-thymidine at a dose 1μ Ci/g 1 hours before the end of experiment. Pieces of testis, liver, kidney and spleen were fixed in Serra's fixative, embedded at paraffin wax and proceeded for routine histological and autoradiographic study.

Bone marrow cells were flushed from femur. Thereafter the cells were fixed in methanol-acetic acid (3:1), dropped on cold slides, air-dried and proceeded for routine autoradiography. The slides were stained with 5% of Giemsa solution. At least 1000

cells were analyzed per animal in the bone marrow slides. The mean ± SD for each group of investigated parameters was calculated. The data from autoradiographs and bone marrow cells were analyzed with Student T-test.

Results and Discussion

Liver

Liver plays a key role in homeostasis maintenance of the whole organism. Detoxication of metabolic products is an expression of its barrier function. The application of polymetal dust resulted mild to moderate changes in the liver structure. At day 60th and to the end of the experiment an increase in Kupfer cells number was registered. In addition inflammation with simultaneous increase of Lymphocyte number around periportal space and bile ducts were seen. In close proximity to inflammation zones an increase in number of binuclear hepatocytes were obvious (Fig 1). In some regions of the liver, the hepatocyte cytoplasm obtained vacuolized appearance as a sigh of lipid degenedarion.

Kidney

The process of detoxication is impossible without the active kidney function. Early changes in kidney morphology were expressed as inflammatory spots in the cortex (Fig. 2) and in the medula of the organ. Malpighian corpuscles in inflammatory zones were rather damaged and at the end of the experiment were completely disturbed. Massive zones of destruction around blood vessels, accompanied with complete tissue distrophy were seen (Fig. 3).

Our data are in agreement with the results about the effect of Al compounds on rat liver and kidney (Nikolova et al., 1994). Probably Al alone or in combination with other metals in industrial dust prominently expresses its negative effect.

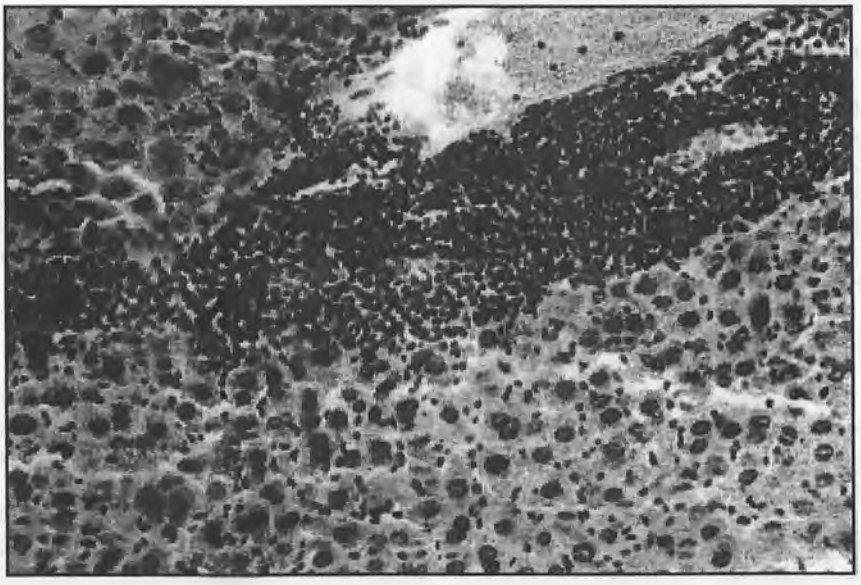

Fig. 1. Section from mouse liver at 60th day of the experiment. Imflammatory zone in close proximity the the periportal spacc. Access of binuclear hepatocytes is obvious. Serra's fixative, Mayer's hematoxilin (× 64)

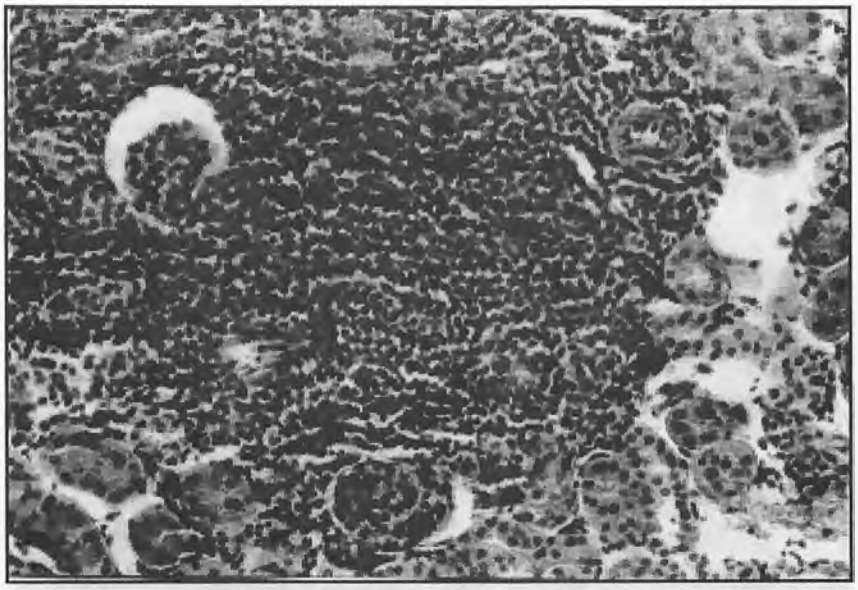

Fig. 2. Section from mouse kidney at 60th day of the experiment. Zone of inflammation in the cortex. Same methods (× 64)

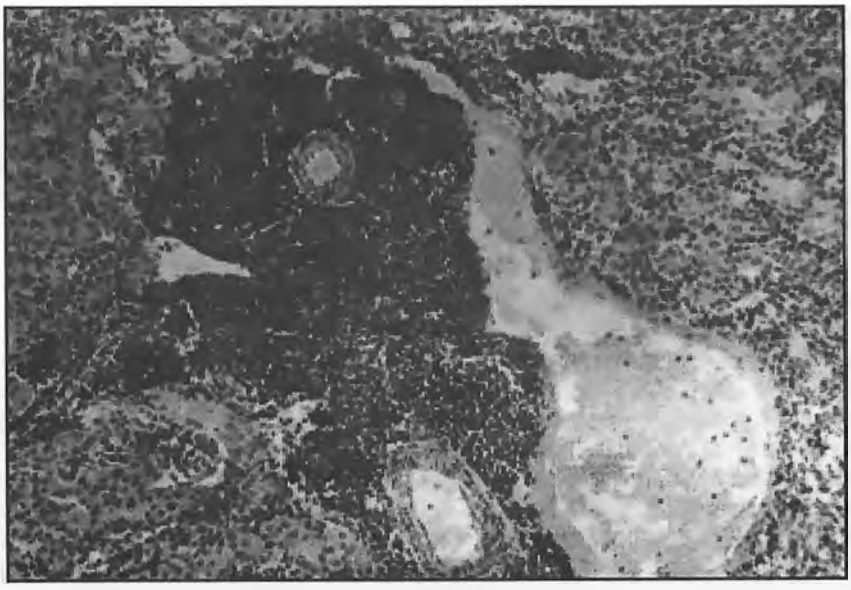

Fig. 3. Section from mouse kidney at 150th day of the experiment. Massive zone of destruction arround blood vessels. Same methods (× 64)

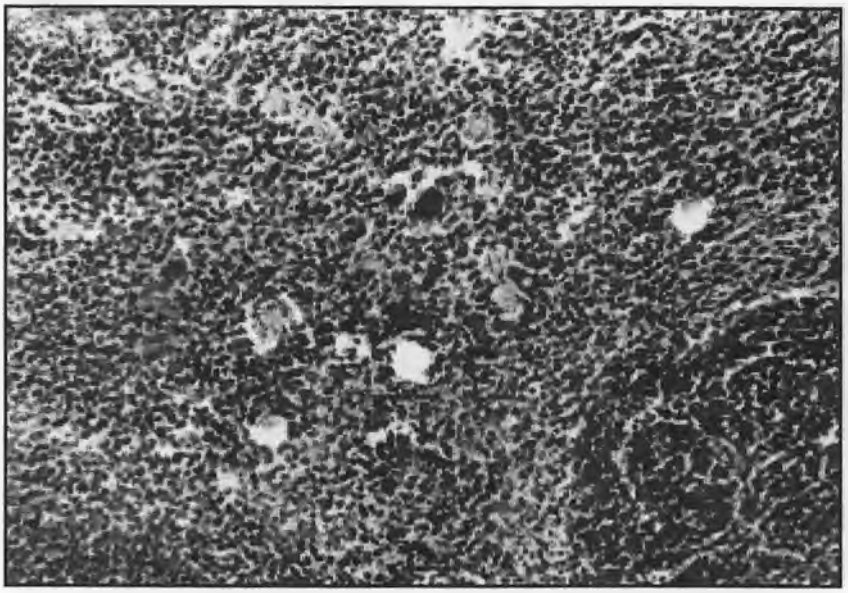

Fig. 4. Section from mouse spleen at 150th day of the experiment. Some megakaryocytes are replaced by vacuoles. Same methods (× 64)

Spleen

As a blood depot, spleen is involved in the protective function. Compared with liver and kidney, the spleen is relatively more resistant to industrial dust toxicity. At 60th day of the study the number of megakaryocytes is increased and the nuclei look condensed and pyknotic. Some megakaryocytes are replaced by vacuoles (Fig. 4). Registered destruction in megakaryocyte morphology may result thrombopeny – a phenomenon that needs further study.

Testis

Any difference in testicular morphology between control and experimental mice was not registered. Obviously the applied doses of industrial dust do not affect the spermatogenic process in mice. The results of autoradiographic study, shown in Fig. 5. demonstrate no difference in spermatogonial proliferation between control and experimental mice. In addition the labelling index of positive control (CP treated) is reduced almost 50% compared with other groups (the same graphic). Reproductive system seems to be the most resistant to industrial dust influence. Similar results we have shown after application of industrial dust from the lead-zink factory in Assenovgrad.

Bone marrow

During the entire experiment the treated mice showed considerable variations in values of thymidine and mitotic indices, while the control mice remained with stable values (Fig. 6 and 7) It is important to consider the significant variations in experimental mice.

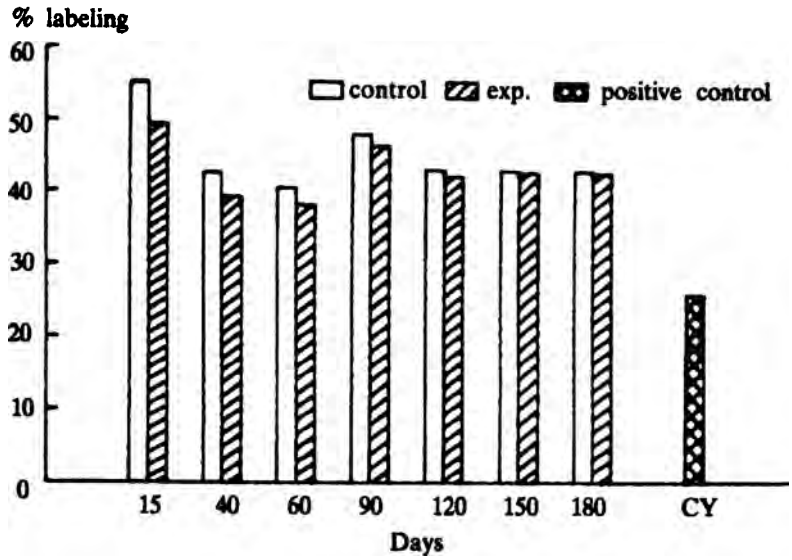

Fig. 5. Effect of polymetal industrial dust on spermatogonial proliferation in control and experimental mice. Obviously there are no substantial differences between contrl and experimental groups. In addition the labelling in CP treated group (positive control) is considerably reduced

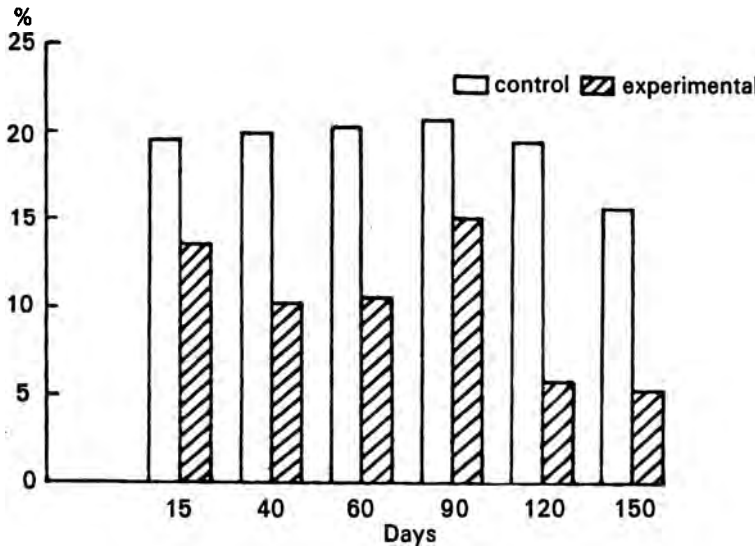

Fig. 6. Effect of polymetal industrial dust on bone marrow cells labelling in control and experimental mice. Compared with the controls, the Thymidine index in experimental groups decreases sharply

In the experimental group as early as on 15th day the percentage of the labelled bone marrow cells was 13.6± 0.00 significantly lower ($p<0.05$) than in the control group. The decrease in bone marrow labeling keeps constant at 40th and 60th days. Thymidine index slightly increases on day 90th but still remain under control values. Sharp decrease of labelling is registered at 120th day and to the end of the experiment (150th day) (Fig. 6). The similar tendency is expressed with the mitotic index showing constantly low percentage starting day 15 to the end of the experiment at 150th day (Fig. 7). On day 90th the increased value in thymidine index does not have any reflection on mitotic index of bone marrow cells. A possible explanation of this result could be asked in a probable delay in transition between G2 to mitosis. At the end of the experiment the value of thymidine and mitotic index statistically does not differ from the data of positive control (data not shown). This is a sign that the studied toxicant (industrial dust) exerts similar cytotoxic effect as CP.

Well expressed correlation was discovered comparing the data about proliferative activity of bone marrow cells with the hematological data [11].

After day 90th, the number of Er of experimental mice increased, due to the presence of immature Er (reticulocytes) in peripheral blood. Consequently, it seems to be related to the physiological adaptation of experimental animals to new conditions with a toxic food. On the 120 day it was registered shock condition for the experimental animals and they showed the lowest values of Hb and Er, while control mice showed to a certain extend stable values. Those variations in the proliferation activity of stem bone marrow cells might be explained with the adaptation of the hematopoiesis to the toxic contents in a food.

In conclusion the polymetallic industrial dust as a waste product of the "Kremikovtsi" factory exerts a toxic effect on morphology of the key organs (liver, kidney, spleen) and on hematopoiesis of white mice during ecotoxicological experiment.

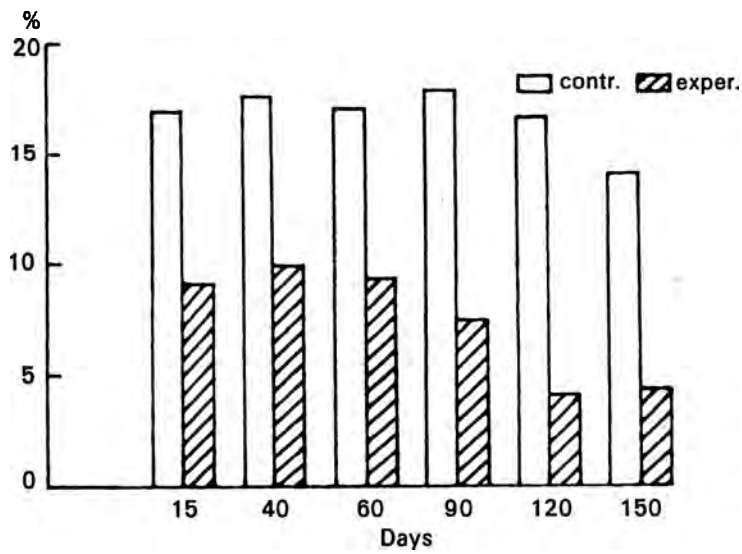

Fig. 7. Effect of polymetal industrial dust on mitotic index of bone marrow cells in control and experimental mice. Mitotic activity of bone marrow cells of experimental groups is strongly depressed

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