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HAEMATOTOXICITY AS AN ISSUE FOR TOXICOLOGY OF CHEMICAL SUBSTANCES AND SOME APPROACHES TO RESOLVE IT

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ABSTRACT. *Haematological studies allowing to assess unfavourable effects on blood system and to develop preventive measures for the reduction of the risk of xenobiotic influence on human health are an indispensable part of complex assessment of toxic properties of pesticides upon hygienic rating. In spite of this fact, publications on the development of haematological diseases in human as the result of contact with pesticides regularly occur in literature. This article summarises and provides some of the peculiarities of experimental studies on the detection of haematological effects of xenobiotics, where the main unfavourable effects that allow assessing potential hazard of the chemical substances by the criterion "haematotoxicity" are emphasised. Approaches to the deep exploration of the effect of pesticides on the blood system and interpretation of the obtained results have been established.*

Key words: *pesticides, haematopoietic system, haematotoxicity.*

The principal task of the complex hygienic rating of pesticides is a comprehensive exploration of the nature of their toxic effect on the body, possible delayed effects and avoidance of the use of the pesticides and their combinations dangerous for human health in agriculture practice. For this purpose, different experimental toxicological studies are performed. They allow to characterise the effect of pesticides on the different organs and body systems of different species of animals, establish "dose-time-effect" relation, determine mechanisms of toxic and specific action, and justify ADI (acceptable daily intake) for human on the basis of the obtained results (considering delayed effects).

Haematological studies have always been the indispensable component in the complex assessment of the toxic properties of pesticides upon their hygienic rating. Assessment of their effect on the blood system was previously based on the exploration of changes in the peripheral blood in laboratory animals. For example, in 60–80s of the previous century, many investigators (N. K. Statsek, 1960; Yu. S. Kahan, 1963; T. N. Panshyna, 1964; V. V. Tarashchuk, 1967; L. M. Sasinovich, 1968; V. I. Matiushyma, 1968; I. N. Krainukov, 1969; A. M. Medovar, 1969; A. V. Bolotnyi, 1973; K. Akhmedzhanov, 1976 and others) noticed changes of the specific findings of the peripheral blood upon the effect of pesticides of different chemical classes [1–10], and they believed that pesticides have no influence on the blood system in general. For example, in 1977, Professor Yu. S. Kahan in his monograph "Toxicology of organophosphoros pesticides" [1] wrote that blood is one of the integral parameters that reflects the effect of pesticide on one or another body system. Therefore, the role of the blood was considered as a secondary one: it was considered as a connector of different organs and systems, and anaemia and disorders in white blood cells were considered as intoxication syndrome.

Along with this, description of cases of haematological diseases in human upon long-term professional and domestic exposure or after acute poisoning with pesticides began to occur in literature: these are pernicious, hypo- and aplastic anaemia, pancytopaenia, agranulocytosis, thrombocytopaenic purpura, porphyria, myeloblastic leukaemia [2–8]. However, experimental studies of the haematopoiesis processes in the bone marrow were isolated and only for those pesticides, for which association with the development of human haematological diseases was proved [2].

Currently, growth of blood and haematopoietic diseases, including malignant, is detected all over the world and in Ukraine in particular [9–12]. Scientists from different countries publish epidemiological data on the development of haematological diseases in human as a result of industrial, agricultural, and domestic exposure to pesticides. These are polydeficit, haemolytic, hypoplastic, aplastic anaemia, agranulocytosis, pancytopaenia,

myelodysplastic syndrome, as well as oncology diseases (non-Hodgkin lymphoma, acute and chronic lymphatic leukaemia, multiple myeloma) [13–17]. Epidemiological studies regarding the development of oncohaematological diseases were conducted throughout all continents, under different conditions of agricultural manufacture, with qualitatively and quantitatively different pesticide burden and considering the different spectrum of competing factors. Recently, in order to prove a reliable association between development of each of types of blood cancer and effect of pesticides, epidemiological data undergo meta-analysis, and risk factors for their development are calculated to confirm carcinogenicity [18–20].

During many years, we also explored the morphological composition of the peripheral blood in the laboratory animals under the effect of many pesticides from different chemical classes in acute, subacute and chronic experiments following different ways of body entry. Upon the analysis of the obtained results, we concluded that not all observed changes following the exposure to some pesticides manifested only at the blood level.

Further conducted complex haematological studies, including exploration of the parameters of the peripheral blood, morphological changes and cytochemical status of the blood cells, as well as haematopoietic processes in the bone marrow and spleen showed that some representatives of pesticides from different chemical classes and their combinations led to the impaired haematopoiesis (Plondrel, Cyclofos, Polycarbacin, Decis, Foxim, combination of Decis and Dursban, Decis and Foxim). Some pesticides showed toxic effect both on the circulating blood and bone marrow, involving cells of different haematopoietic lineages at different levels of proliferation, differentiation, and maturation, and some (Plondrel and combination of Decis and Foxim) on their early stages: up to pluripotent progenitor cell [21–26].

Upon the expert evaluation of the dossier of the companies for new molecules of pesticides, proposed for approval in Ukraine, in order to assess the nature of their toxic effect on their body and justification of human ADI, careful analysis of possible manifestations of their haematological action is obligatory performed. As a result of conducted expert evaluation, it was established that many of the substances led to the disorders in red and white blood to any extent. Among these substances, there were representatives with toxic effect on the bone marrow resulting in the depression of one or another haematopoietic lineages, as well as with double or triple mechanism of haematological disorders development. Effect on haematopoiesis was explored only for small amount of pesticides leading to the disorders in haematological parameters and mechanism of their haematotoxic effect was established. Nature of changes in the bone marrow was essentially judged on the basis of visual analysis of histological slices of the bone marrow without quantitative assessment of haematopoiesis processes.

Considering the above, we think that the principal task of haematologists working in the field of pesticides and other chemical substances toxicology is a deep and comprehensive assessment of their effect on the blood system. Experimental and expert evaluation of pesticides requires more thorough approach to the analysis of the results of haematological studies aimed at the exclusion of the possibility to use substances which may lead to the haematological diseases in human. Long-term experience of experimental and expert work allowed us to develop approaches and criteria for the assessment of haematological effect of pesticides [27].

At the first stage, screening of haematotoxicity of pesticides by the parameters of peripheral blood in acute, subacute, and/or subchronic experiments is performed. According to the requirements of Good Laboratory Practice (GLP) for screening of pesticide haematotoxicity it is foreseen to perform enough standardised set of haematological tests (content of haemoglobin, red blood cells count, haematocrit level, coefficients of red blood, platelets count, total number of white blood cells and white blood cell differential). At the same time, not enough data are available upon primary assessment of the haematological action to perform complete blood count. Results of calculation of the number of reticulocytes and analysis of morphological disorders in the cells of red and white blood are also required. At the stage of screening assessment of haematotoxicity in terms of the parameters of the peripheral blood, it is possible to establish. Whether the detected changes are really redistributive and reflecting condi-

tion of other organs and body systems, or they have a toxic effect on the circulating blood. At the same time, screening assessment results allow only to suppose possible effect on the haemotopoiesis. Therefore, in case of detected changes showing haematotoxic effect, appropriate assessment of haematotoxicity requires further more deepened studies (both following acute and chronic exposure to xenobiotic). The aim of the extended analysis is to establish the nature and mechanism of pesticide toxic effect both in the circulating blood and in the bone marrow, spleen and other haematopoietic organs, namely, whether the substance has an effect on haematopoietic processes, changes synthesis of haemoglobin (leading to the impaired porphyrin metabolism, met- and sulfhaemoglobin formation, and other disorders). At the same time, differentiation of disorders requires involvement of the data of biochemical, pathomorphological tests and experiments for the exploration of mechanism of haematotoxic effect on the blood system. With the knowledge of manifestations of haematological diseases in human, it is required to find an opportunity to establish the diagnosis of haematological disease in animals to extrapolate experimental data on a human.

Upon examination of blood in laboratory animals, it is required to consider that per cent of changes in the parameters is not so high and does not fully reflect the deepness of lesions in the haematopoietic organs. Previously, we provided a comparative analysis of changes in haematological findings in laboratory animals and in humans following acute poisoning with organophosphoros pesticides (chlorophos, karbophos, dimethyl dichlorovinyl phosphate) in isotoxic doses [21]. It was shown that changes in the parameters of the peripheral blood in humans and rats are unidirectional, however, they are more pronounced in humans. This is due to the fact that mature rodents (rats, mice, guinea pigs) is characterised by genetically determined protection mechanism in the form of activation of extramedular haematopoiesis in the spleen [28, 29] that is partially compensated blood parameters upon the effect of haematoxic agent. Upon the depression of haematopoiesis in the bone marrow of laboratory animals, degree of the development of extramedular haematopoiesis in the spleen and other haematopoietic organs (liver, lymph nodes, kidneys, lungs) depending on the degree of the depression. In this regard, one must consider peculiarities of reactivity of animal haematopoietic system upon extrapolation of experimental data to humans.

It should be also considered that contrary to the histological assessment of the effect of the substance on the haematopoietic organs, quantitative analysis of haematopoiesis processes is more conclusive. Analysis of myelogram/splenogram allows to conduct more objective assessment of the effect of xenobiotics on the processes of haematopoiesis: calculation of the cellular elements of all haematopoietic lineages at different stages of their differentiation and maturation, ratio of cellularity of one or another haematopoietic lineage in respect to each other, number of mitoses, as well as morphologically changed or destructed cells. Furthermore, it is required to calculate the total number of myelocariocytes and calculate the absolute content of each of the cell type. Calculation of myelogram/splenogram allows not only to understand possible mechanism of action of the substance on the blood system, but also to assess all stages of haematopoiesis processes — from stem cell of the bone marrow to the pool of mature circulating cells of the peripheral blood.

Accumulated experience of experimental studies and expert assessment allows us to conclude that from haematotoxicity point of view, the most dangerous are pesticides, following the action of which:

— haematological effect at the level of low doses, especially in case of small dose variance (increment) is manifested;
— there is no distinct dose-response relation;
— changes progress over time;
— haematotoxic effect is manifested at the later terms of the experiment and/or do not resolve following discontinuation of the exposure;
— there is a significant selectivity of action on the blood system, as well as species- and gender-specific sensitivity;
— depression of haematopoiesis, especially at early stages of cellular proliferation — at the level of steam and multipotent progenitors;
— development of pronounced extramedular haematopoiesis is observed;
— involution of the spleen and the thymus is observed;
— pronounced haemolysis of red blood cells in the circulation and, especially, those that

inhibit erythropoiesis in the bone marrow is detected;
— impaired porphyrin metabolism, that may lead to porphyria, diagnostics and treatment of which is highly challenged;
— as well as substances with double and triple (several) mechanisms of haematotoxicity development;
— combined preparations containing active ingredients that have a unidirectional mechanism of haematotoxicity, especially if they impair haematopoietic processes.

It is known that diseases of chemical origin, including haematological, have a lot of own peculiarities contrary to classical diseases. They frequently have a non-typical course, since different mechanisms of the toxic effect on the body are involved in the basis of their development. This is due to the fact that the substance absorbed into the blood is distributed by the blood to different organs and body systems. Molecules and their parts formed during metabolism, may cause disorders in different organs and body systems, in particular, affecting both cells of circulating blood and haematopoietic organs. The latter is the most dangerous in terms of possible development of haematological diseases in humans as a result of the exposure to such substances. Differential diagnostics and treatment of such conditions is unconditional and provides significant difficulties for clinicians.

In this regard, for correct assessment of haematotoxic effects that may occur following the exposure to pesticides, haematologists working in the field of toxicology should be trained. Researcher haematologist-toxicologist should have an appropriate knowledge of outstanding features of the blood system of laboratory animals and human, nature of possible manifestations of haematotoxic effect of pesticides. For this purpose, professionals educated in the field of laboratory and clinical diagnostics of human haematological diseases should be involved. Only a highly professional haematologist-toxicologist may provide appropriate interpretation of the results of haematological studies following the exposure to any of the chemical substances and perform correct extrapolation of experimental data to humans.

Conclusions

1. Study of haematotoxicity of chemical substances, including pesticides, is a topical task of current toxicology.

2. Exploration of haematotoxicity of xenobiotics should be stage-by-stage. If haematotoxic effects are detected at the screening stage, it is required to foreseen further more comprehensive and deepened experimental studies aimed at the establishment of the nature and mechanism of toxic effect of xenobiotics both on circulating blood and haematopoietic processes.

3. Considering the negative effect of the chemical substances on the human blood system, it is required to develop more current approaches to experimental studies of haematotoxicity and extrapolation of data from animals to humans.

4. In order to resolve the above issue, special education of haematologists-toxicologists is required.

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