

## The Possibility of Application of Carbonic Anhydrase Activity Index at Early Diagnostics of Haemolytic Processes

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**ABSTRACT.** To define additive effect of the mixture of pesticides the use of real clinical parameters is required. The approach of early diagnostics of haemolysis at the combined toxycoses was elaborated. The investigation showed that the above mentioned index fixed the beginning of haemolysis much earlier than other haematological parameters. © 2019 Bull. Georg. Natl. Acad. Sci.

**Key words:** carbonic anhydrase, addictivity, early diagnostics, life resource

In modern clinical practice early diagnostics of pathologies of different ethiology is still a problematic question. Statistics of recovery shows straight coorelation of efficacy of medical actions and stage of starting diagnosis of pathology. The most absorvable dependence is seen at curing of cancer diseases and some systematic pathologies of different ethiologies.

We studied combined toxycoses in both sharp and chronical tests, while dealing with questions of pesticide control. The tests were conducted on laboratory rats, rabbits and sheep within the Institute of Sanitary and Hygieny, Ministry of Health, Georgia in the period of 1999-2006.

The aim was to investigate and find the coefficient of additivity for different pesticides which are used in agrochemical scheme of plant protection simultaneously and for a short period of

time. Combinations of copper and zinc in pesticides according to their application for agricultural purposes were studied.

Different clinical indices of blood, such as quantity and diameter of erythrocytes, hemoglobin, alanine aminotransferase – AAT, aspartate aminotransferase – ACT, thymol, quantity of leucocytes, thrombocytes, etc., also, indices of milk quality: density fat content, acidity, protein and caseine – wool quality of sheep: fraction component, artificial and real length of fibre, tonine, strength of flock.

When determining the additivity of different combinations of chemical substances the proper selection and evaluation of clinical signs of organism as well as highlighting specific toxic effects present a particular difficulty. That obviously refers to multiplied experiments, as

during vivisection experiments lethality is a basic criterion for the evaluation of a level of toxicosis.

The experiments were carried out on thirty sheep (40-48.5 kg live weight), twenty-eight lambs under six months of age (about 21.5 kg live weight), seventy two laboratory rats and three hundred thirty-six pubescent chinchilla rabbits. These studies confirmed the complicacy of determination of a dominating toxicodynamic link for the evaluation of systemic impact on pesticide mixture.

In view of the fact that the pesticides under study were predominantly characterized by the hemolytic action, particular attention was paid to the evaluation of additivity on this parameter. However, assessment standard criteria were not informative enough to determine the toxicodynamic effect.

To provide a more accurate assessment of additivity, we have developed a method for the early detection of hemolysis. The method authored by Gligvashvili G. A. and Gegenava O. B. was patented as invention #876 (28 Sep 1997). The essence of this method lies in the comparison of the ratio of carbonic anhydrase in the supernatant and in laked blood.

$$K = \frac{E_1}{E_2} \cdot 100,$$

where

$K$  – Hemolysis coefficient;

$E_1$  – Amount of carbonic anhydrase in the supernatant of blood;

$E_2$  – Amount of carbonic anhydrase in laked blood.

In the specialist literature sometimes synonyms of carbonic anhydrase – carbonate dehydratase and carbonate hydrolase – are used. It is a ferment, which catalyzes the reversible reaction of the hydration of carbon dioxide according to the following pattern:



In plasma the reaction proceeds slowly, while in erythrocytes ten thousand times faster.

Consequently, carbonic anhydrase is an important link in the maintenance of the acid-alkaline equilibrium, transport of carbon dioxide, etc. Amount of carbonic anhydrase in blood is significantly increased, but changes drastically in the case of certain pathologies. For instance, the increase of enzyme activity is observed in various types of anemia, bronchiectasis, pneumosclerosis, etc., while there is a marked decrease of activity in renal acidosis, hyperthyroidism, etc.

When determining carbonic anhydrase activity by Brinkman's method, in the norm it is equal to 2-2.5 standard units, where doubling the speed of a catalyzed reaction is defined as the unit of enzyme activity. In the norm the carbon dioxide hydration reaction time is 110-125 seconds.

The largest amount of carbonic anhydrase is concentrated in erythrocytes and an increase in the amount of this enzyme in the supernatant indicates hemolysis. Hydrolysis is a multistage process and at the early stage of pathology during swelling of erythrocytes until their destruction, carbonic anhydrase is ejected into blood serum while preserving the erythrocytes.

Studies have shown that when multiplying the toxic effects of the studied compounds, there was observed a shift in the rate of enzyme until there occurred a shift in the rate of erythrocytes.

It should be noted that the level of reliability for determination of the shift (the carbonic anhydrase activity rate in the supernatant) is directly dependant on the time frames for clinical trials.

In our experiments, laboratory animals had been given pesticide mixtures in a dose of 1/20 LD<sub>0</sub> five times a day for two weeks, while clinical trials had been conducted for thirty-five days.

The results of these experiments showed that the coefficient of hemolysis reacted to toxicosis about twelve or fourteen days earlier than the indicator of the number of erythrocytes and was the most reliable criterion for the evaluation of additivity of the effects of mixtures of pesticides.

The remaining clinical features not so accurately determined the synergistic effect of the mixture what, from a practical point of view, is of great importance for the specification of the permissible norms of the presence of these pesticides in agricultural products.

The quest for the key links for identification of the start of pathological process can be effective not only in the study of the toxicodynamics of various chemical compounds.

It can be assumed that the absence of tangible results in the study of various systemic diseases,

including cancer, is a consequence of the methodological inadequacy of modern approaches.

From this perspective, it is interesting to study the biological systems that fall outside statistical validity (for example, in determining LD50 and LD100 of various chemical compounds, as well as with the lethal effects of radiation).

The absence of lethal effect is an important foundation for studying a particular object with a view to identifying the reasons for sustainability. In this regard, the coefficient of hemolysis at carbonic anhydrase can be informative.

*ადამიანისა და ცხოველის ფიზიოლოგია*

## კარბონჰიდრაზის აქტივობის მაჩვენებლის გამოყენების შესაძლებლობა ჰემოლიზური პროცესების ადრეული დიაგნოსტიკის დროს

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პესტიციდების ნარევის ადიტიური ეფექტის განსაზღვრა სარწმუნო კლინიკური პარამეტრების გამოყენებას მოითხოვს. ჩვენ მიერ შემუშავებულ და დაპატენტებულ იქნა ჰემოლიზის ადრეული დიაგნოსტიკის მეთოდი კომბინირებული ტოქსიკოზის დროს. კვლევებმა აჩვენა, რომ აღნიშნული მაჩვენებელი ჰემოლიზის დაწყებას აფიქსირებდა გაცილებით ადრე, ვიდრე სხვა ჰემატოლოგიური პარამეტრები.

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