TOXICOLOGIC EVALUATION OF INDIVIDUAL CHEMICAL COMPOUNDS AND THEIR COMPLEX MIXTURES USING A MOTILE CELL TEST OBJECT

R. I. Kayumov, A. P. Es'kov,
I. M. Aref'ev, V. G. Lappo,
and Yu. S. Rotenberg

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The toxicologic and hygienic monitoring of materials and objects, and sanitary and ecologic monitoring of the environment necessitate evaluation of the combined effect of individual chemical compounds on biological objects. The use of physical and chemical methods capable of selectively determining concentrations of chemical compounds is not always effective, for the discovery of the whole spectrum of individual chemical substances is a complicated and laborious task, more especially becuase the biological action of chemical compounds cannot be reduced simply to summation of the effects of their individual components. A solution to this problem can be found by using bioengineering measuring systems, capable of evaluating the simultaneous action of a whole range of factors. Systems using subcellular organelles, various types of cells, and cellular structures as test objects are known [1, 5, 8]. The use of motile cells as test objects is a very promising development, for the effect of individual chemical compounds or their mixtures is thereby transformed into a change in the physiological function (motility), characteristic of biological objects of this kind. This function can be transformed into an electrical signal, which can be processed by traditional technical systems.

The aim of this investigation was to study the possibility of quantitative prediction of the toxicity of chemical compounds and their complex mixtures with the aid of a bioengineering system, using bovine sperm, preserved until the experiment in the frozen state, as the test object.

EXPERIMENTAL METHOD

The principle of operation of the bioengineering system is based on analysis of the change in dependence of motility of spermatozoa on time. The parameter of motility m is defined as $\tilde{m} = \alpha \cdot C_m \cdot \bar{V}$, where α is a coefficient depending on the design of the apparatus, C_m the number of motile cells, and V the average modulus of velocity of cell movement. The method of preparation of the working solutions and a block diagram of the apparatus used to determine $\tilde{m}$ were described previously [2]. In each experiment four or five samples of the control and experimental solutions were used. For each sample of working solutions the dependence $\tilde{m} = f(t)$ was obtained and the average value of this function (t_{av}) was calculated

$$t_{av} = \frac{\sum_i \tilde{m}_i \cdot i}{\sum_i \tilde{m}_i},$$

where i is the serial number of the estimation of motility in the sample. The average value of the function characterizes the average duration of motility of the spermatozoa in suspension. The dependence $\tilde{m} = f(t)$ for one control sample is shown in Fig. 1. As a measure of toxicity of the test substance or mixture of substances, we used an index of toxicity I_T:

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TABLE 1. Values of Inhibiting Concentrations and Toxicometric Parameters

Compound	$\log \frac{1}{C_{i_{50}}}$ (M – m)	$\log \frac{1}{LD_{50}}$	$\log \frac{1}{MAC_{WZ}}$
Methanol	0,1±0,08	0,76	3,81
Ethanol	0,25±0,18	0,76	1,66
Caprolactam	0,56±0,25	1,74	4,05
Vinylpyrrolidone	0,96±0,06	1,91	—
Tetrahydrofuran	1,10±0,21	1,38	2,86
Acrylamide	1,11±0,15	2,55	—
Methyl methacrylate	1,45±0,7	1,11	—
Dichloroethane	1,85±0,24	1,93	3,99
Cyclohexanone	1,91±0,14	1,74	3,99
Phenol	2,02±0,16	2,31	4,27
Acrylic acid	2,13±0,21	2,42	4,16
Epichlorhydrin	2,23±0,21	2,74	4,97
Ethylenimine	2,99±1,23	3,46	6,34
Formaldehyde	4,37±0,22	1,87	4,78

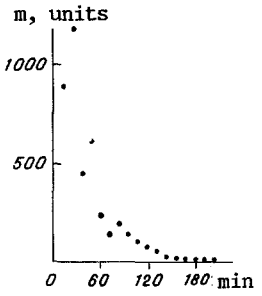

Fig. 1. Dependence of motility of spermatozoa on time in glucose–citrate medium.

$$I_T = \frac{\bar{t}_{av}^{expt}}{\bar{t}_{av}^{cont}} \times 100\%,$$

where $\bar{t}_{av}^{expt}$ and $\bar{t}_{av}^{cont}$ are average values for several experimental and control tests respectively. I_T was determined for different concentrations of test substances, and from the dependence $\log \frac{1}{C_i} = f(I_T)$ (Fig. 2) $C_{i_{50}}$ (the concentration of the substance inhibiting cell motility by 50%) was calculated. Comparison of values of $C_{i_{50}}$ of the compounds and their toxicometric parameters (the half lethal dose for rats (LD_{50}), and the maximal allowable concentration for air of the working zone (MAC_{WZ}) was done by the linear regression method. As the test substances we chose compounds migrating from medical polymer materials and also ethanol. Values of LD_{50} and MAC_{WZ} were taken from [3, 4, 7] and converted into molar units. Since the toxicometric parameters and $C_{i_{50}}$ for different compounds differ by several orders of magnitude, in this paper we used logarithms of reciprocals.

EXPERIMENTAL RESULTS

Table 1 gives values of concentrations inhibiting motility of a suspension of spermatozoa and toxicometric parameters of the compounds tested. It will be clear from Fig. 3 that a relationship exists between the inhibitory activity and acute toxicity, expressed by the equation:

$$\log \frac{1}{LD_{50}} = 0.82 + 0.76 \log \frac{1}{C_{i_{50}}}$$

with a coefficient of correlation r = 0.81. A marked deviation of LD_{50} was found for formaldehyde from this dependence. The possibility cannot be ruled out that for compounds in whose mechanism of toxicity an important role is played by reflex reactions, the relationship between the data obtained in vitro and toxicity for the whole organism may be differ-

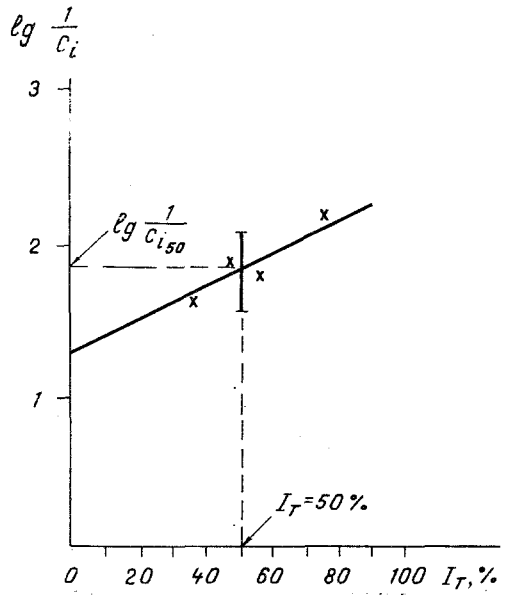

Fig. 2. Determination of $C_{i_{50}}$: dependence $\lg\frac{1}{C_i} = f(I_T)$ for dichloroethane.

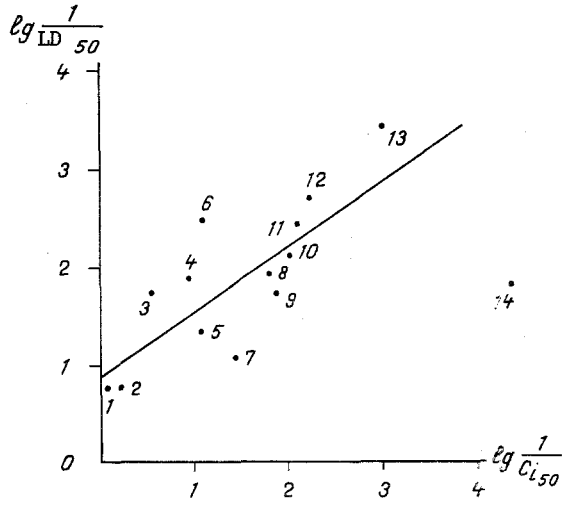

Fig. 3. Correlation between inhibitory activity and acute toxicity.

ent in character. The relationship between values of $C_{i_{50}}$ and MAC_{wz} is expressed by the equation

$$\log\frac{1}{MAC_{wz}} = 2.54 + 0.97\log\frac{1}{C_{i_{50}}}$$

and the coefficient of correlation r = 0.76. The average difference between the calculated values of LD_{50} and those given in reference books for the compounds tested (except formaldehyde) is by a factor of 3.1, which does not lie outside the usual limits of scatter for results obtained by different laboratories [9]. The average difference of the calculated values of MAC from those in the reference books is by a factor of 6.2, which also is perfectly acceptable. On the basis of results obtained in experiments with individual chemical compounds, we tested 54 aqueous extracts from materials and articles used in medicine. Parallel studies of these extracts were undertaken by the usual toxicologic methods [6]. In 52 cases the results coincided, and in two cases the method used proved to be more sensitive than the standard methods.

The results are thus evidence of the effectiveness of this rapid method of toxicologic screening both of individual chemical substances and of medical polymers. The possibility of storing the test object for a long period, the simplicity of preparation of the cell suspension, and the high freedom from interference of the method used to record motility all enable the system developed for this purpose to be recommended for use not only in the laboratory, but also in industry: when assessing various batches of raw materials, choosing optimal ways of sterilizing objects, and when introducing minimal changes into the formula of a material. It must be emphasized that the system as developed allows not only mammalian spermatozoa to be used as the test object, but also other motile micro-objects (microorganisms, protozoa, and so on), depending on the specific aim of the investigation.

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