THE USE OF BULL SPERMATOZOA SUSPENSION FOR EVALUATION ACUTE AND LOCAL TOXICITY

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Bull spermatozoa is one of perspective mammalian cells for creation in vitro cytotoxicity tests. Test method which can be used to predict toxic effects (acute toxicity and local toxicity) on complete mammalian organism was developed.

The test method operation principle is based on the influence of the examined solution or extraction from material on the change of motility of bull spermatozoa. The energy dependence of all processes in the cell justifies the assumption that the action of chemical agents on the reproductive cells causes changes in the energy metabolism which, in their turn, result in spermatozoa motility changes. The measuring parameter is the motility of bull spermatozoa suspension m, which is proportional to the moving spermatozoa concentration c_m and average modulus of the cell velocity v:

$$m = c_m v$$

The bull spermatozoa suspension motility $m = m(t)$ for reference and examined samples is measured during period of time till motility becomes close to zero (fig.1).

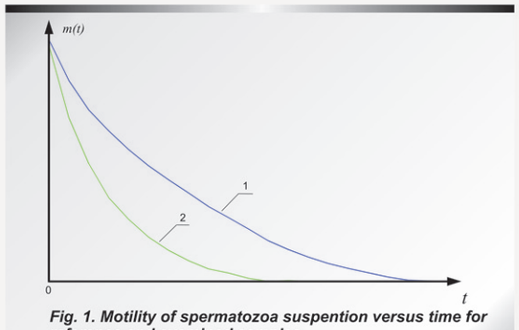

Fig. 1. Motility of spermatozoa suspention versus time for reference and examined samples.
1- reference sample; 2-examined sample.

Two characteristics of suspension motility function $m = m(t)$ are used as endpoints. They are: the value of average weighted suspension motility time

$$t_{\text{av}} = \frac{\int_t m(t)t\,dt}{\int_t m(t)\,dt}$$

and summary suspension motility

$$S = \int_t m(t)\,dt\,.$$

Then the result of examination is received in the form of toxitciy index I_t equal to ratio of correspondig endpoint values for examined and reference samples. They are:

$$I_t^{t\text{av}} = \frac{t_{av}^{exam}}{t_{av}^{ref}} \cdot 100\%,$$

where t_{av}^{exam}, t_{av}^{ref} - average weighted values of suspension motility time for examined and reference samples, and

$$I_t^S = \frac{S^{\text{exam}}}{S^{ref}} \cdot 100\%,$$

where S^{exam}, S^{ref} - summary suspension motility for examined and reference samples.

Using integral characteristics of cell suspension motility function $m = m(t)$ as endpoints increases test method susceptibility and reprodusibility.

The correlation coefficient between the chemical compounds toxicity parameters in the case of the complete mammalian organism and the case of spermatozoa suspesion was determind for the substances migrating from medical application polimers (Table). The logarithms of the inverse values of the rat's half-lethal dose of the investigated compounds administered intra-abdominaly $\lg \frac{1}{DL_{50}}$ and their concentrations causing 50 percent reduction of spermatozoa motility avarage time $\lg \frac{1}{C_{I_t^{tav}\,50}}$ were compared.

The correlation coefficient r = 0.83 was got. This result corresponds with the results of MEIC study that mammalian basal cytotoxicity tests are relevant for predicting the human acute toxicity of chemicals.

Test method was used for evaluation toxicity of extracts with complicated compositions. In practice it permits for a particular kind of production on the base of cytotoxicity experimental result to predict toxic effects (acute toxicity and local toxicity) on complete mammalian organism. To evaluate prediction model, the limiting values I_t of It which in the parallel experiment on the complete mammalian organism reveals the toxic effects of the examined aqueous extracts must be found.

If cytotoxicity can be measured without examined sample dilution, the limiting values of toxitciy index I_t can be determined as follows. It is necessary to plot the function of correlation coefficient $r = f(I_t)$ between the results of the investigation on complete organism and on spermatozoa suspension to the value of the toxicity

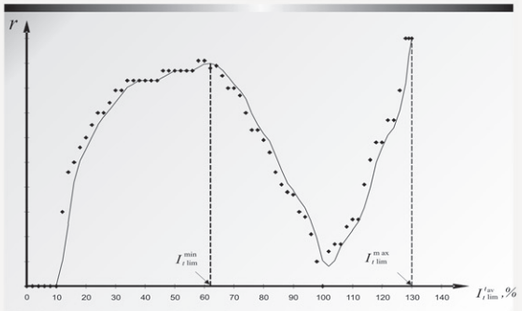

Fig. 2. Correlation coefficient between the results of the investigation on complete organism and on spermatozoa suspension versus value of the toxicity index assumed to be the limiting value. Data for disposable syringes

index assumed to be the limiting value (fig. 2). This being the case, the results of investigation on both biological models are presented in alternative form. Function $r = f(I_t)$ is plotted for ranges $0 < I_t \leqslant 100\%$ and $100\% \leqslant I_t < \infty$. The values of toxicity index I_t for which the extremum of function $r = f(I_t)$ is true correspond to the limmiting values of the toxicity index. The two limiting values of the toxicity index correspond to the suppressing effect ($I_{t\,\lim}^{\max} > 100\%$) and stimulating effect ($I_{t\,\lim}^{\min} < 100\%$). With the toxicity index in the range $I_{t\,\lim}^{\min} \leq I_t \leq I_{t\,\lim}^{\max}$ no toxic effects on complete mammalian organism can be predicted. This procedure was used to evaluate prediction models for polymeric medical products. For example, for disposable syringes the following prediction model was got: if $70\% \leqslant I_t^{tav} \leqslant 120\%$ predict no acute toxicity, otherwise predict acute toxicity. Predictive value (r^2=0,96).

If dilution of examined sample is necessary to measure citotoxicity, the limiting value of toxitciy index I_t can be determined as follows. With this purpose in view it is necessary to plot the function of toxitciy index $I_t = f(C)$ versus value of the dilution C for a lot of examined samples. Among the samples which do not reveal toxicity effects on complete mammalian organism must be found the sample with highest cytotoxity. The limiting value of toxitciy

Table. Inhibiting concentrations and toxicity parameters

Compound	$\lg \frac{1}{C_{I_t^{tav}\,50}}$, mole^{-1}	$\lg \frac{1}{DL_{50}}$, mole^{-1}
Methanol	0,1±0,08	2,73
Ethanol	0,25±0,18	0,76
Caprolactam	0,56±0,25	1,74
Acetone	0,29±0,03	1,08
Vinyl pyrrolidone	0,96±0,06	1,91
Tetrahydrofuran	1,10±0,21	1,38
Acrylamide	1,11±0,15	2,55
Methyl methacrylate	1,45±0,7	1,11
Ethylene dichloride	1,85±0,24	1,93
Cyclohexanone	1,91±0,14	1,74
Phenol	2,02±0,16	2,31
Acrylic acid	2,13±0,21	2,42
Epihlorohydrin	2,23±0,21	2,74
Ethyleneimine	2,99±1,23	3,46
Formaldehyde	4,37±0,22	1,87

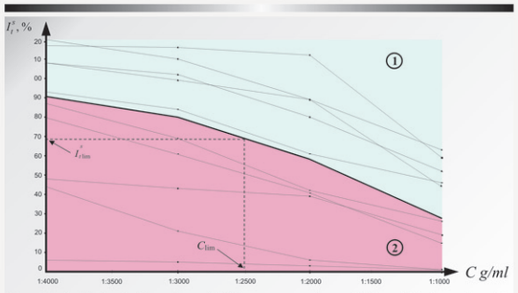

Fig. 3.Toxitciy index $I_t = f(C)$ versus value of the dilution for the sample with the highest cytotoxicity among the samples which do not reveal toxicity effects on complete mammalian organism. 1- zone of the samples which do not reveal toxicity effects on complete mammalian organism. 2- zone of the samples which reveal toxicity effects on complete mammalian organism. Data for shampoos.

index $I_{t\,\lim}$ is set arbitrary. Then from the function $I_t = f(C)$ corresponding dilution value $C_{\lim}$ is found (fig. 3). For the samples with the toxicity index $I_t \geq I_{t\,\lim}$ for the dilution $C_{\lim}$no toxicity effects on complete mammalian organism can be predicted. This procedure was used to evaluate prediction models for different perfumery and cosmetic products. For example for shampoos the following prediction model was got: I_t^S>70% for C=(1:2500), predict as no skin corrosion, otherwise predict as skin corrosion. Predictive value (r^2 = 0,92).

In practice measuring process of toxitciy index I_t is rather simple. Frozen cells stored in liquid nitrogen in Dewar vessel are used that allows to ensure the unlimited storage and reduce time for work preparation up to 10 min. It is a cheap and accessible biological material which is easy to produce thanks to well developed processes of arttificial fertilization and readily availiable from the rejected hereditary-inadequate stock. The examined and referece solutions are prepared as follows. Granule of the bull's frozen sperm is defrosted in glucose-citrate medium at the temperture of 40C after which 0,2 ml of the defrosted spermatozoa suspension is added to 1 ml of the examined and reference samples. The sell concentration is adjusted to make period of expirement not longer than 3 hours. Glucose-citrate medium (4 g of glucose, 1 g of sodium citrate, 100 ml of distilled water) is used as reference medium. The isotonicity of the examined solution is adjusted by adding dry glucose and sodium citrate. During the entire experiment the temperature maintains 40C. Disposable glass capillaries of 25 mcl volume are applied as chambers. The motility of spermatozoa suspension is measured by specialy developed toxicity analyser (Fig. 4). The operating principle is based on real time automatic computer microscopic videoimage analysis of spermatozoa suspension. The values of bull spermatozoa suspension motility for control and examined mediums are measured in equal intervals and accumulated, till motility becomes close to zero. Then toxitciy indexes I_t^S, I_t^{tav} are calculated.

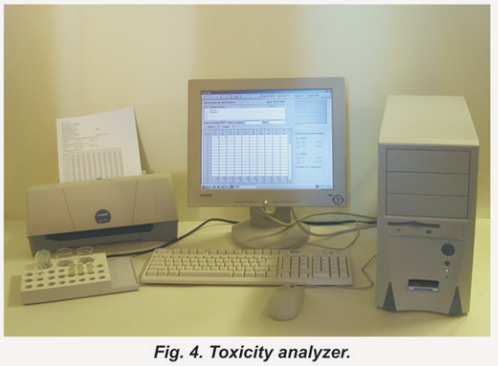

Fig. 4. Toxicity analyzer.

In Russian Federation test methods for evaluating acute and local toxicity for medical products, for products made of polimer and other materials, for perfumery and cosmetic, for home used chemical production, for water and air samples are approved. Now the test methods and toxicity analyser are used by factories, regional Departments of State Sanitary and Epidimiological Supervision and scientific laboratories.