**PANBIORA – WEB-TOOL.**

**The purpose of developed Web-tool is the predictive analysis (extrapolation) of toxicity profile for substances released from a biomaterial during its biodegradation.**

**The developed software provides possibility to predict the level of toxicity of soluble factors, released both by ordinary dissolution and by biodegradation of the material with the participation of biological factors (for example, perycellular proteolysis).**

**An obvious prerequisite for the model construction is the assumption that biomaterials with obvious high toxicity (for example, LD50 < 10 mg / ml, t < 12 h) are to be excluded from the following testing already at the stage of preparing implantable materials using standard in vitro cytotoxic tests.**

**At the same time, for biomaterials with initially low and not obvious toxicity we preliminarily implement the analysis that should uncover whether significant difference with the control (obviously non-toxic) substance is present. This analysis will be carried out using program "Statistic", and further step using program "Hepatotoxicity test" will be applied for substances with low but significantly reliable (statistically) toxicity.**

**The advantage of this method is that it allows to identify / predict effects that are impossible or difficult to register using standard methods of toxicological analysis in vitro and even in vivo: for example, if the cumulative toxic effect of substances, released inside recipient organism from a biomaterial, is so negligible that cell death in significant / reliable values will be observed only after days or even weeks for cell culture (that principally contradicts experimental methods). In this situation the program 3 gives possibility to predict the LD50(30), as well as to predict the concentration of a substance that can cause a toxic effect (it opens possibilities for prediction of hepatotoxic and carcinogenic effects at the level of the recipient organism).**

**In this program, we partially used the approach developed for estimation of hepatotoxicity of some pharmacological substances in order to calculate the kinetics of cell death. This approach describes the cell death under the influence of metabolites produced from the primary (test) substance (drug) processed by hepatocytes. The concentration of this drug is known and constant over time.**

**The program provides possibility to define specific cell death pattern typical to the substance. At the first phase, the coefficient of natural cell death can be calculated (using parameters of control culture), after that at the second phase, parameters of cell death under the influence of tested substance can be defined. Additionally, if the initial concentration of the toxin is determined by any chemical (photometric, etc.) technique, the LC50 can be easy calculated (however, even in the case of unknown initial concentration some function of this parameter still can be calculated). The final task of elaborated program is to analyze the experimental data using a suitable mathematical model describing the toxic effect in order to determine the parameters of the model and extrapolate the toxic effect for long period for evaluation of LC50 or its equivalent (for example, specific coefficient defined by the area of tested biomaterial seeded with target cells). Then it is possible to recalculate the toxic effect for other conditions (in vivo + other surface area of the biomaterial that releases the toxin).**

**During further modeling we are going to adapt the model for the following cases:**

**1) discriminatory analysis of the effects of both the toxin itself and its metabolite (as already proposed by us for hepatocellular spheroids). Toxic properties can be intrinsic for: 1. a substance (primary) released from tested material; 2. a substance and its metabolites 3. the cellular metabolite of this substance (in the Yeon model, the hepatocyte metabolite of tested drug);**

**2) account for the dynamic change in the concentration of a possible toxin in the measuring system due to its diffusion and processing by hepatocytes (due to the small volume of liquid extracted for analysis from microfluidic system);**

**3) calculation of the biodegradation rate of the material when the areas of its contact with cells and biological fluids are known (preliminary calculations have been already made and demonstrated by us earlier).**

**Since the range of measurements using the aplyed model is limited by a few values, we offer here several options to demonstrate how hepatocytes can process a substance to form toxic metabolites, that in its turn affects the viability of target cells (see please attached files with initial values for paracetamolum, diclofenak etc.)**

**EXAMPLES.**
**The values should be inserted manually (we will adapt data input by files during a few weeks).**
**Testing species concentration*,uM – "T"**
**Rate of cell natural death**, 1/h - "gamma"**
**Reference time for LC50 evaluation,h - "beta"**
**Concentrations in % to initial - "Cexp"**

## ***Acetaminophen :***

**"T": 5000.000,**
**"gamma": 5.300e-02,**
**"beta": 1.656e-02,**
**"mu": 2.696e-05,**
**"t_ref": 1.50,**
**"LC50":3.476e-308,**
**"Cexp":**
**100.000,100.000,100.000,94.200,78.100,56.900,43.100,32.800,27.000,16.100, 0.000, 0.000, 0.000**

## ***Verapamil***

**„T": 100.000,**
**"gamma": 5.300e-02,**
**"beta": 2.117e-02,**
**"mu": 2.982e-03,**
**"t_ref": 1.50,**
**"LC50":3.476e-308,**
**"Cexp":**
**[**
**100.000,99.300,83.800,71.300,54.400,35.300,13.200, 0.000, 0.000, 0.000, 0.000, 0.000, 0.000**

## ***Diclofenac***

**"T": 300.000,**

"gamma": 5.300e-02,
"beta": 4.117e-02,
"mu": 1.093e-03,
"t_ref": 1.50,
"LC50":3.476e-308,
"Cexp": [ 100.000,100.000,93.400,83.800,54.400, 5.880, 0.000, 0.000, 0.000, 0.000, 0.000, 0.000, 0.000