Biological activity tests of some Poznik's d.o.o. informed products: Stickers, Energy cards and Informed boards

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INTRODUCTION

With information technology we can change the matter (water, air ...). It positively affects our well being, mood and health. By informing it is possible to neutralize negative radiation and information pollution, which contrary to measurable chemical pollution, can not be controlled yet. Cellular phones have harmful effects because of heir's radiation, operating at fine levels, damages the gene structure. The long-term effects of such radiation will only be revealed in time. The pollution caused by scanning the bar code is harmful. Research has shown that the information the code reader sends to the product has harmful effects because it changes the nature of the product. There is not much talk about information pollution, but in the highest academic institutions, a number of and we will have to wait for some of their findings.

"Now we are only dealing with information's," explain Vili Poznik. Our greatest desire is to be able to influence to the nature of the information with the information technologies, and that the person who received these positive changes in the environment would live better and be physically, mentally and spiritually healthier, more acceptable to him and others. "This means that you would be a first-aid man choose an informational panel that would nullify bar codes, live water could protection for the computer; so there would be beneficial changes for humans, for plants, for animals. «We also managed to inform bee stickers - this dot-based therapy is known. We managed to capture the character of the beehive poison, capture it in code and transfer it to labels. »They are very effective because we managed to com-bine two technologies, api therapy and information technology Poznik". In addition, we successfully informed: stickers, various cards and boards, including Apit for Varoa (*Varroa* destructor, parassita of Apis mellifera).

Informed Cards "Poznik d.o.o."

Informed boards "Poznik d.o.o."

MATERIAL AND METHODS

1. Material

In the experiments carried out we analyzed the biological efficiency:
1.1. Energized signs
1.2. Informed cards
1.3. Informed boards
the following cell types were used: Human macrophage (TL-1) - non-transformed, CaCo-2 and HeLa transformed (= cancerous).

2. Methods

2.1. Growth Index (GI)

Cell suspension (1 ml) was distributed over micro titer plates, which were incubated for 3 days at 37 ° C and 5% CO2. After the incubation, the tiles were fixed with glutaraldehyde, washed with a saline solution and stained with a solution of 2% purple violet in 20% ethanol for 10 minutes. Then, the dye was flushed, the cells were blunted, dried, and measured OD at 595 nm. GI = OD after 3. Day / OD of initial number of cells.

2.2. Tumorigenicity index In vitro (IT In vitro)

Cell suspension (1 ml) was partitioned into micro titer plates with 0.001 mM $CaCl_2$ and 1.5 mM $CaCl_2$. Cells were incubated for 3 days at 37 ◦ C and 5% CO_2. After the incubation, the tiles were fixed with glutaraldehyde, washed with a saline solution and stained with a 2% Purple crystal in 20% ethanol for 10 minutes. The dye was then drained, dried and measured at 595 nm OD. From the mean values we calculated the Tumorigenicity index: IT in vitro = OD at 0.001 mM$CaCl_2$ / OD at 1.5 mM $CaCl_2$.

2.3. Apoptosis and apoptosis index

The suspension of the cells, untreated and / or treated, were sediment and suspended in 100 µl of PBS, which was added to 100 µl of AO (Acridin orange) / EB (Etidium bromide). We took 20µl of suspension and placed it on the slide, covered with a roof slide and counted colored cells (brown) under fluorescence. From the number of colored and uncolored cells we calculated the percentage of apoptotic cells and hence the Apoptosis index (AI).

2.4. Determination of NO value

Take 50 µg of supernatant treated / untreated cells and add 50 µl of Gries reagent (1% sulfanilamide in 2.5% H_3PO_4 and 0.1% Naphtyl-thylene-diamino hydrochloride in distilled void). Incubate all of them for 30 minutes at 37 ° C and measure OD at 530 nm.

RESULTS

1. Testing of information stickers on the inhibition of CaCo-2 cell growth and their's effect on tumorigenic potential under in vitro conditions.

In the first part of the experiment, an informed sticker was affixed to the bottles: xx-yy-C (POZNIK) for 24 hours / 37◦ C. In the second part of the search, stickers were

labeled on the bottles and illuminated with a 45W bulb / 24 hours, compared to unexposed cells with labels LIGHT. At the end of the exposure, the cells were trypsinized and further cultured in 96-hole plates so that after 3 days of incubation we could calculate: GI (Growth index) (10% FCS) (No. Cells after 3 days / Cell initial Number); CPD = (Log N1 - Log NO) / Log 2 where N1 is Number of Cells after 3 days, N0 Initial number of Cells and IT in vitro (Tumorigenicity index in vitro) (Cell number at 0.01 mM CaCl2 / Cell number at 1.00 mM CaCl2). In the experiments performed after a single exposure (24 hours / 37St.C), we obtained the following figure of percent inhibition of cell growth (GI and CPD) and percent of Inhibition of IT in vitro inhibition (Reduction of tumorigenic potential. The results are shown in TABLE 1.

TABLE 1.

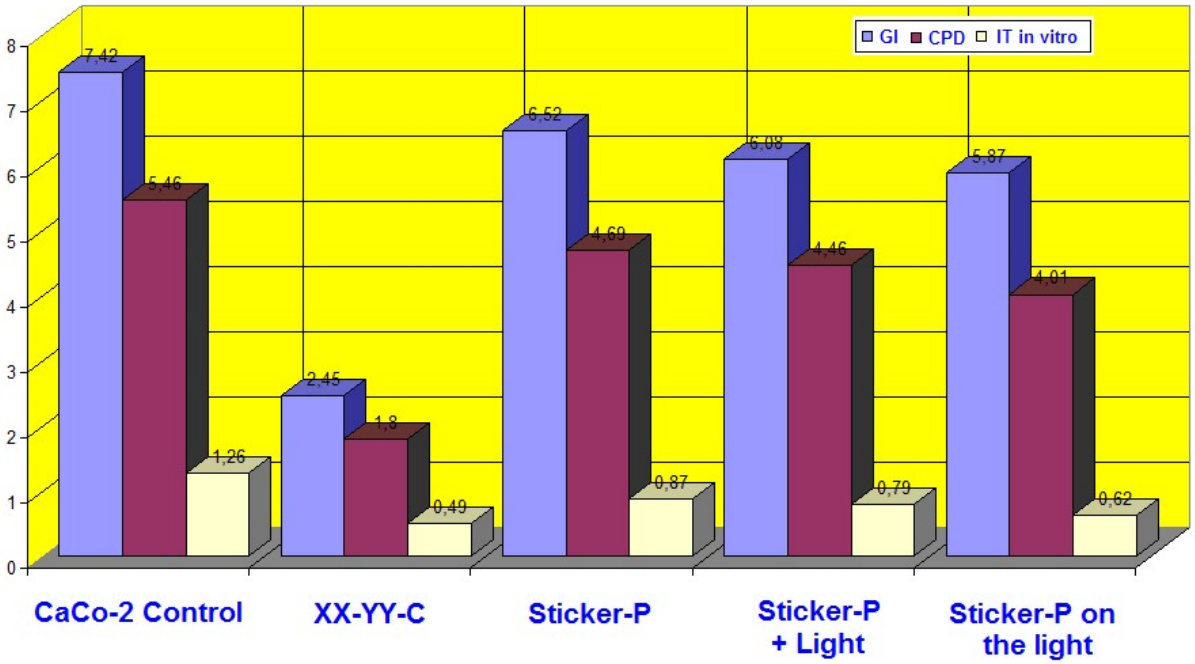
	GI (10%)	**CPD**	**IT In vitro**	**% OF INHIBITION**
CaCo-2	7.42	5.46	1.06	0
+ Sticker xx-yy-C-(Poznik) (24 h/37°C)	2.449	1.802	0.49	67
+Sticker	6.52	4.69	0.87	12
+Sticker + light (24 h/37° C)	6.084	4.46	0.79	18

From the experiments we have made, we can summarize the following:

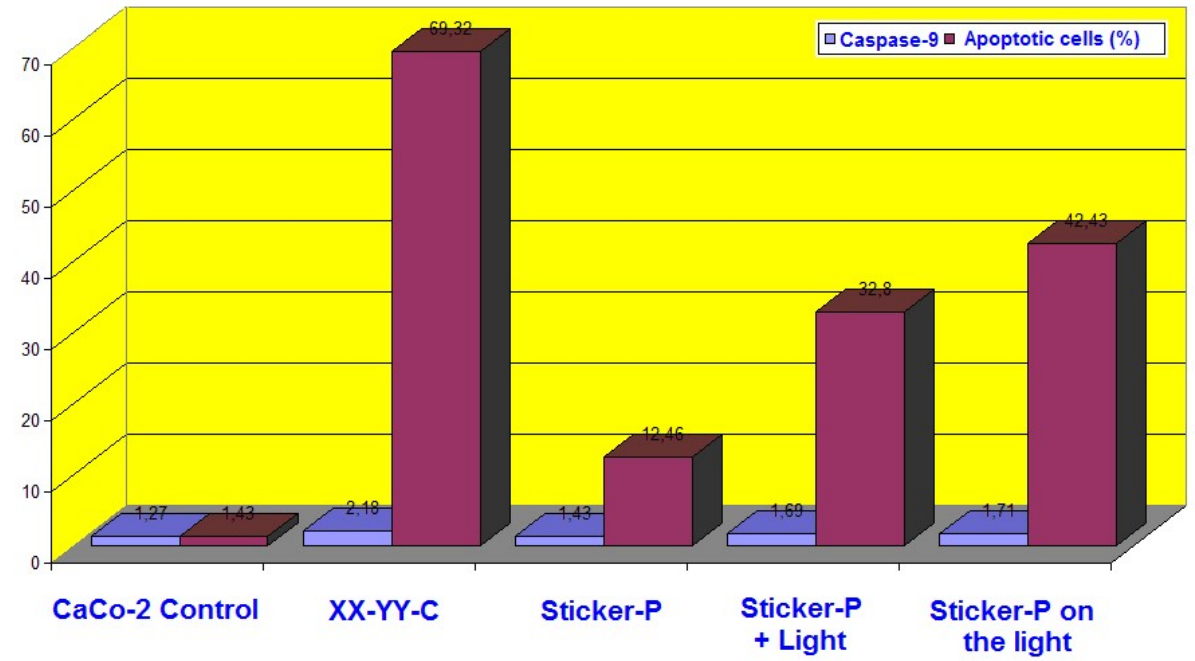
1. The notified label (xx-yy-C- (Poznik) affixed to the CaCo-2 cell bottle by mediating the information leads to a decrease in GI and CPD, thereby decreasing the growth of CaCo-2 cells, as well as a reduction in their potential of tumorigenicity. In the following, it would be important to determine how many exposures would cause 90% inhibition, as well as repeat the same experiment on CaCo-2 micro tumors.

2. In analyzing the effect of exposure on an informative label, we found that an Increase in percent of inhibition (GI, CPD, and IT in vitro) occurs, but 18%, up to 2% in control, is not the most significant.

Effect of Stickers (Poznik d.o.o.) on the growth (GI, CPD) and Tumorigenicity potentital (IT in vitro) of CaCo-2 cells.



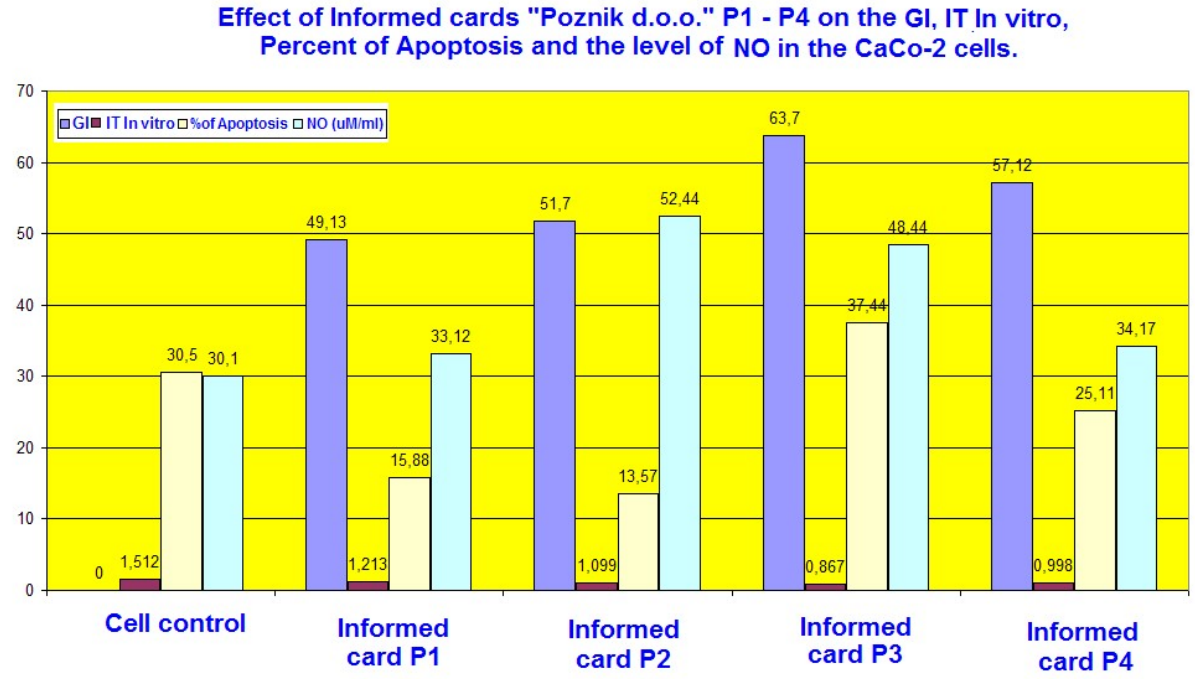
Effect of Stickers (Poznik d.o.o.) on the Caspase-9 level and Percentage of Apoptotic cells of CaCo-2

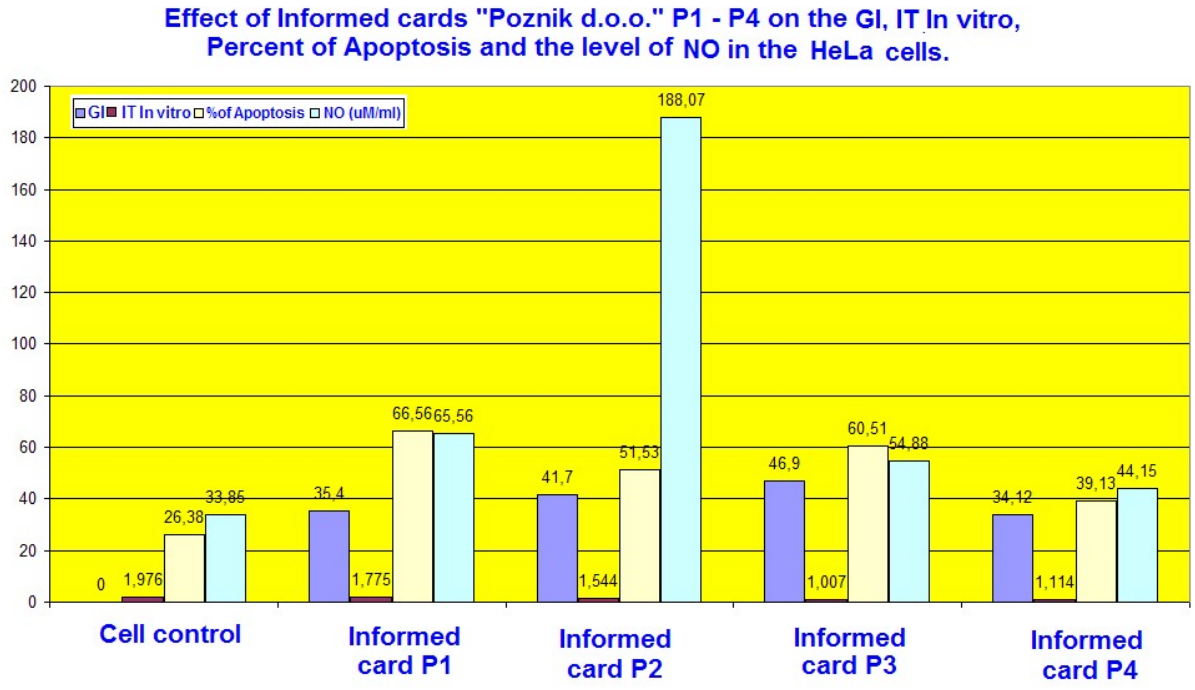


Conclusion: An informed label xx-yy-C- (Poznik) can be an important potential tool in the treatment of surface experimental tumors in vivo (mice, rats), although the nature of the information in the labels is unknown.

2. Testing the effect of exposure to CaCo-2 and HeLa cells to different Informed cards: P1, P2, P3 and P4 to the level of Growth Index (GI), Tumorigenicity index In vitro (IT in vitro) , Apoptotic Cells and level of Nitric Oxide (NO)

In the experiments we performed, we used 4 bottles of CaCo-2 cells and 4 HeLa cells, which were placed on different cards Poznik: P1, P2, P3 and P4 for 1 to 3 days at 37° C.





CELLS:	% of Inhibition of GI	IT in vitro	% of Apoptosis	NO (µM / ml)
CaCo-2 - Control	---	1.512	30.5	30.1
+ Card P1	49.13	1.213	15.88	33.12
+ Card P2	51.7	1.099	13.57	52.44
+ Card P3	63.7	0.867	37.44	48.44
+ Card P4	57.12	0.998	25.11	34.17
HeLa - Control	---	1.976	26.38	33.85
+ Card P1	35.4	1.775	66.56	65.56
+ Card P2	41.7	1.544	51.53	188.07
+ Card P3	46.9	1.007	60.51	54.88
+ Card P4	34.12	1.114	39.13	44.15

From the experiments we have made, we can summarize the following:

1. Inhibition of GI was the most effective with P3 card (63.7% CaCo-2, 46.9% for HeLa cells)
2. In inhibition of IT in vitro, the most effective was card P3 (CaCo-2 0.876 / 1.521 in control; Hela 1.007 / 1.976 in control)
3. In the effects on percent of apoptosis, the best was again card P3 (CaCo-2 37.44 / 30.5 in control; Hela: 60.51 / 26.38 in control)
4. In the effects on NO quantity in the supernatant, the best was card P2 (CaCo-2 52.44 / 30.1 in Control; HeLa: 188.07 / 30.1 in control)

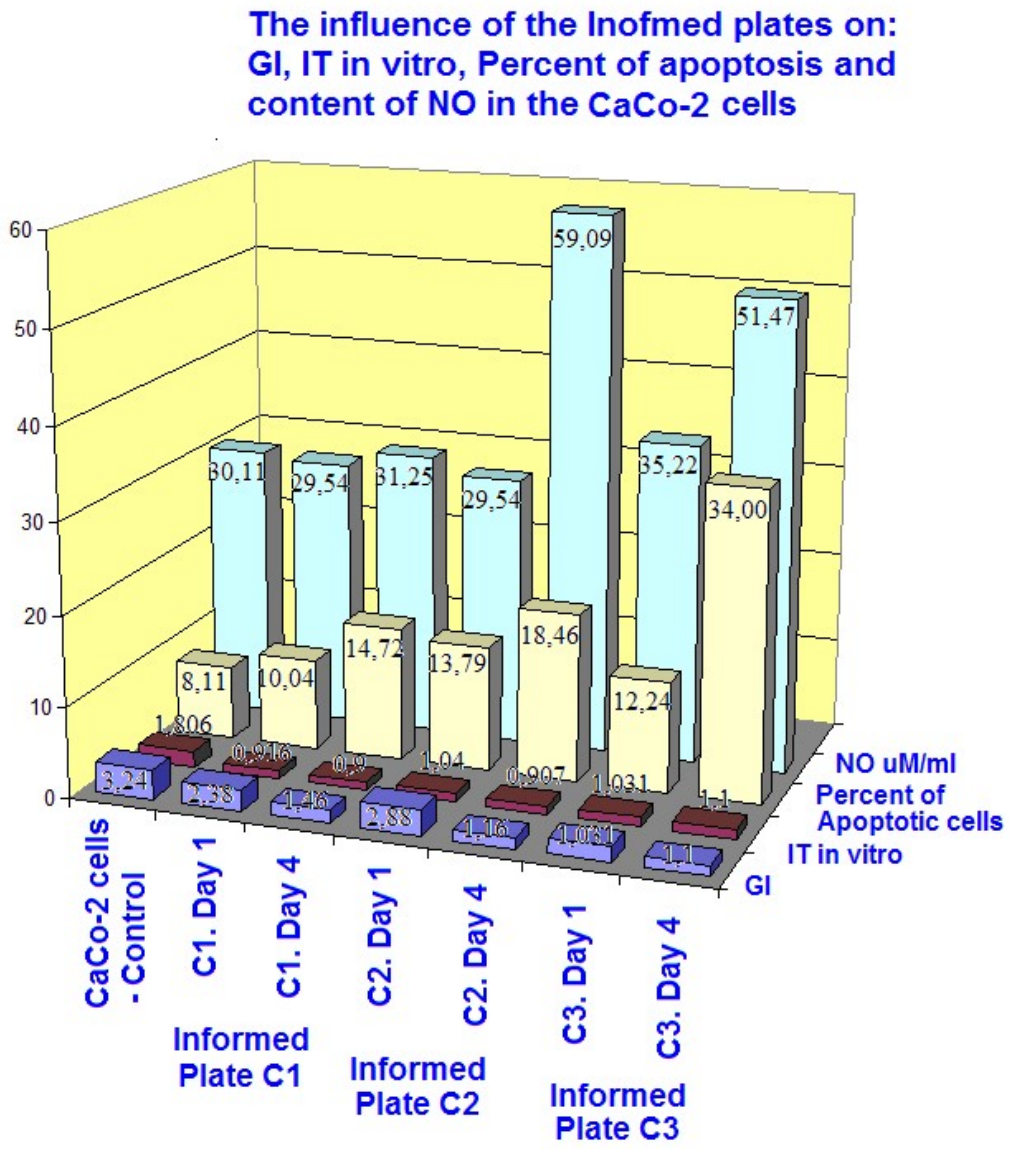
We can conclude: For successful operation, it would be useful to combine Information's from cards P2 and P3 card into one, if possible.

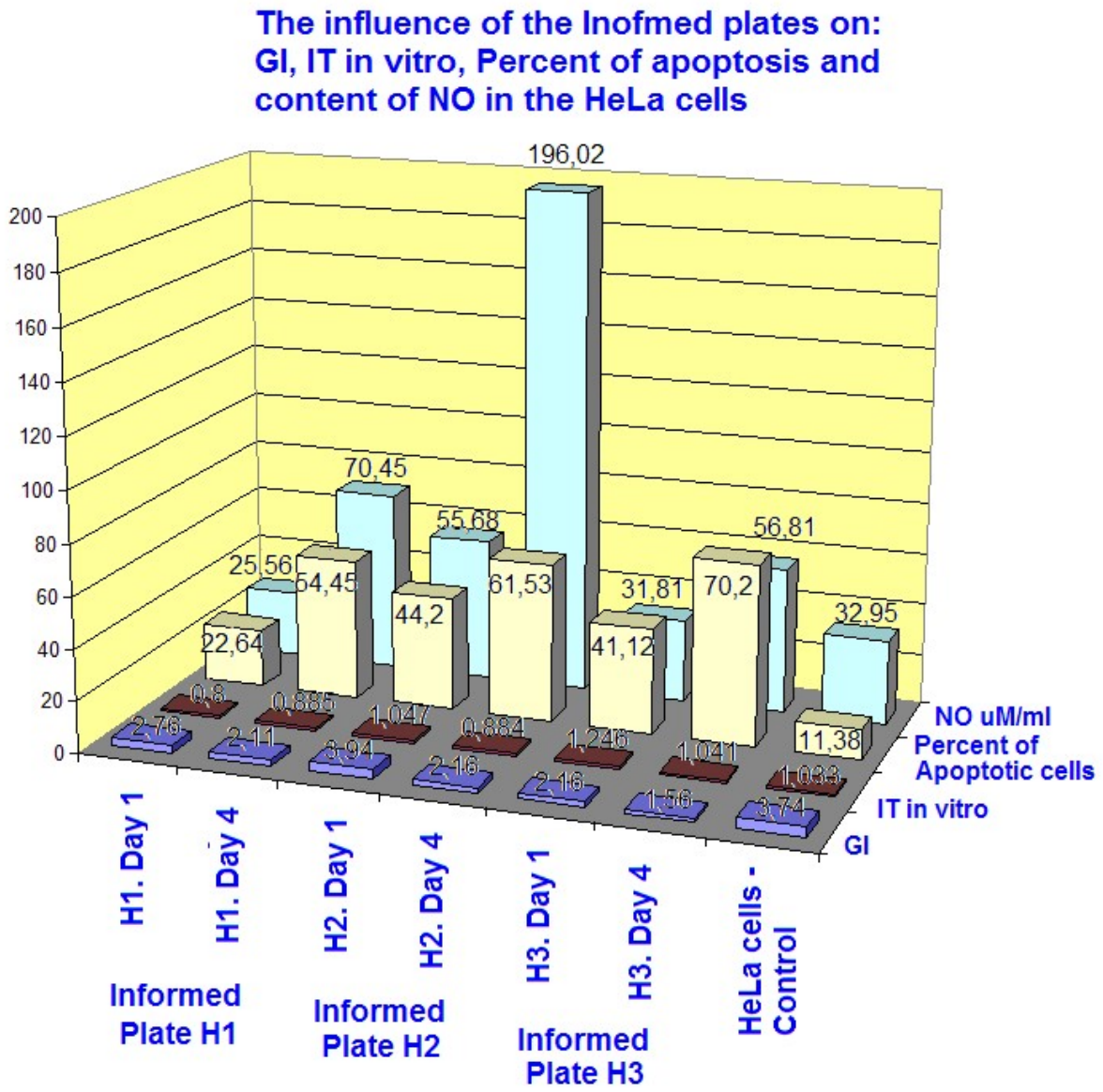
3. Testing the effect of the exposure of CaCo-2 and HeLa to the sign panels: C1, C2 and C3 to the level of Growth Index (RI), In vitro (IT) Inhibit Index, Apoptotic Cells and Nitric Oxide (NO)

In the experiments we used 4 bottles of CaCo-2 and HeLa cells, which were highlighted on the C1, C2 and C3 sign panels for 1 to 4 days at 37 ° C. After exposure, supernatants of the cells were separated in which the NO and the cells we have they were marketed and given GI and IT in vitro.

CELLS:	% of Inhibition of GI	IT in vitro	% of Apoptosis	NO (µM / ml)

CaCo-2 - Control	---	1.806	8.11	30.1
+ Panel C1	45.1	0.9	14.72	31.25
+ Panel C2	35.8	0.907	18.46	59.09
+ Panel C3	52.5	1.10	34.00	59.47
HeLa - Control	---	1.976	19.38	33.85
+Panel H1	56.7	0.800	54.45	70.45
+ Panel H2	57.80	0.884	61.53	196.02
+ Panel H3	41.80	1.041	70.20	56.81





From the experiments carried out to test the bioavailability of various Informed Plates that we exposed to the CaCo-2 and HeLa cells we can summarize:

1. Growth index (GI)
   Thus, after the exposure of CaCo-2 and Hela to cells of various informative Plates, RI is reduced. The best in both cases was the Informed Panel 3 (1.1 / 3.2 in the control of CaCo-2 cells and 1.56 / 3.74 in the control of HeLa cells).
2. Tumorigenicity index in vitro (IT in vitro)
   it was demonstrated that after CaCo-2 cell exposure to the information plate 2 The IT in vitro was 0.907 / 3.24 in the control, and after the HeLa cell exposure To the informed plate 2 the value of IT in vitro was 0.884 / 1.033 in control.
3. Percent of apoptotic cells
   Research on the percentage of apoptotic cells after exposure of CaCo-2 and HeLa cells to various information plates' results in an increase in the percentage of apoptotic cells. For CaCo-2 cells, this was Information Panel 2 (18.46 / 8.11 in Control of CaCo-2 cells); for HeLa cells, this was the Informed Panel 3 (70.2 /

11.38 In the control of HeLa cells)

4. Nitric oxide (NO)
   Analysis of the NO value after exposure of the CaCo-2 and HeLa cells to various Informed plates showed that the highest NO values in the Informed Panel 2 (59.09 / 30.1 were in the control of CaCo-2 cells; 196.02 / 32.95 in the control of HeLa cells)

We can conclude: For successful operation, it would be useful to combine information's from the Informed Plate 2 and 3 into one, if possible, because such a new Informed Panel would give completely new features, not only against Varoa, but also against transformed (= cancerous) cells.