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The study of the cytotoxic effect of disinfectants

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ABSTRACT

The toxicity of individual disinfectants has been studied in vitro using human cell cultures (HT-29 (epithelial-like cells of colon adenocarcinoma), HEK 293 (human embryonic kidney cells)) to create a model for assessing the toxicity of residual amounts of disinfectants that can enter milk for a person. Standard tests have been used to assess cell viability and amount: methyl tetrazolium (MTT) test, neutral red cell staining (NRP), and sulforhodamine B (SRB) test. Disinfectants have a dose- and time-dependent cytotoxic effect on human cell cultures. IC50avg (concentration of the drug that suppresses a certain cell function by 50%) of disinfectants based on the effect on cell cultures (average value) is Biodez – 117.29 ±14 µl/l, Blanidas – 389.25 ±20.83 µl/l, Virkon-S – 343.04 ±28.04 µl/l, Neochlor – 473.82 ±30.16 µl/l, Phan – 56.71 ±7.05 µl/l, Chlorination – 343.28 ±27.26 µl/l, Chlorinated lime – 117.35 ±9.44 µl/l. Mean toxic doses for cell cultures are lower than the mean lethal dose (based on literature data) for rats and mice by gastric administration. The novelty is that determining the cytotoxicity of disinfectants in vitro using human cell cultures can significantly reduce the number of animals for establishing LD_{50} during the registration procedure of new agents, making it possible to make preliminary conclusions about the toxicity of substances at the stage of chemical screening, preliminary hygienic regulation, identify target organs of toxic influence.

Keywords: toxicity, cell culture, in vitro, disinfectant, genotoxicity

INTRODUCTION

Any new chemical compound, regardless of its intended purpose, shall be characterized in terms of its possible toxicity and biological activity. In addition, toxicological testing of drugs in preclinical trials shall ensure obtaining a reliable toxicological assessment **[1]**. Since Ukraine joined the European Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes on May 2, 2017, it is now clear that conducting all types of toxicological research on animals is not appropriate. The list of limitations for using laboratory animals for preliminary evaluation of disinfectants includes ethical issues, but economic and time costs are not less important, which significantly increase the cost and length of research **[2]**. In vitro methods are widely accepted in toxicology and widely used for the screening and classifying of chemicals **[3]**. Meanwhile, there is a sufficient number of standardized methods, in particular, using cell cultures of different organ natures, which allow obtaining adequate information for assessing the impact of xenobiotics on cell metabolism **[4]**, **[5]**, **[6]**. The variety of test systems is extraordinary, and more than 170 commercial organizations offer in vitro toxicity testing services covering mechanisms such as genotoxicity, hepatotoxicity, cardiotoxicity, and immunotoxicity **[7]**, **[8]**.

As for the dairy industry, scientists are developing methods to evaluate the quality of equipment disinfection **[9]**. In particular, the adenosine triphosphate bioluminescence method to determine bacterial contamination of surfaces and a test procedure for evaluating the cleanliness of milking equipment **[10]**.

Studies of structure-cytotoxicity relationships in many analogues of compounds can provide more detailed insights into the mechanisms of their action at the molecular and cellular levels **[11]**.

Various model systems have been created and improved to study all types of toxicity at the cellular level **[12]**, **[13]**.

We have investigated basic toxicity, which corresponds to a greater extent to acute toxicity at the microorganism level. Many markers for assessing the degree of cell damage in culture have already been developed, but it is obvious that the violation of even one of the cellular functions inevitably entails a negative impact on the overall viability of the monolayer after a certain time **[14]**. This facilitates the task of researchers significantly, as it involves, at least at the first evaluation stage of an in vitro toxicological study, the use of a limited set of cell cultures (most often standard, common lines) and a few simple indicators of cell viability **[15]**, **[16]**, **[17]**.

All of the above fully applies to disinfectants and their toxicity tests.

An integral component of safe food technology is the observance of good hygienic practices (GMP) during the entire process chain, an important component of which is the sanitation of process equipment **[18]**.

Modern disinfectants used for sanitizing milking and technological equipment of milk processing enterprises are multicomponent. They include not only active substances of disinfectants but also surfactants, stabilizers, etc. **[19]**.

The use of such means allows you to combine cleaning and disinfection in one operation and reduce the duration of sanitary treatment and water consumption. It is worth noting that it is necessary to carry out sanitary treatment of milking and technological equipment under technological instructions. In case of violated regimes of the final cleaning of disinfectants from the surfaces of technological equipment, the probability of xenobiotics entering milk and dairy products increases significantly **[20]**.

As a result of the use of detergents and cleaning disinfectants during technological processes related to the sanitation of food production equipment, unregulated by-products can be formed due to the interaction of chemical and organic substances, get into food products and cause food poisoning in people **[21]**. Metabolites of disinfectants can have carcinogenic and non-carcinogenic effects, in particular, affect endocrine disorders in the body. Understanding the consequences of such impacts on public health is of urgent importance to society and public officials responsible for the safety of drinking water and food **[22]**. By-products from the use of chlorine-containing disinfectants in the dairy industry inhibit iodine absorption in humans and contribute to the formation of metmyoglobin, which is a high risk, especially for children **[23]**.

In the available literature over the past 5 years, 9 groups and 36 types of such substances have been identified. Moreover, there is a statement that these compounds can be more toxic than the disinfectant itself. Such compounds in drinking water are of particular concern to scientists, as new disinfectants have recently emerged that require detailed investigation into the formation of toxic metabolites **[24]**.

Compliance with the rules of sanitary processing of equipment used for the manufacture of food products is very important for the safety of the final product since the residues of detergents and disinfectants can affect both the health of a consumer and the control tests for the presence of other harmful substances in the products. Thus, the residual amounts of such substances affect the reactions of the tests (BRT MRL; Delvotest SP-NT MCS; Eclipse 100) during the screening of antibiotics in goat milk **[25]**.

This problem in Ukraine is practically unexplored due to the lack of effective methods for determining micro concentrations of disinfectants in milk.

Improvement of methodological approaches during veterinary and sanitary control of food safety indicators and study of the possible negative impact of small doses of foreign chemical substances, including disinfectants and detergents and disinfectants, on human health are of important scientific and practical importance. Preventing residual amounts of disinfectants, detergents, and disinfectants in milk as a result of technological processes of its production and the corresponding control of the presence of these agents in milk is an urgent issue and requires in-depth scientific research and justification.

The paper aims to determine the cytotoxicity of low concentrations of individual disinfectants in vitro as a model for determining residual amounts of disinfectants that may enter milk during its production technology.

Scientific Hypothesis

We hypothesized that disinfectants with chemicals in their composition show dose-dependent cytotoxicity to human cell cultures. Their toxicity depends on the chemical nature of the disinfectant components and correlates with LD50 values obtained from tests with laboratory animals.

MATERIAL AND METHODOLOGY

Samples

Disinfectants – Biodez R (the active substance is polyhexamethylene guanidine hydrochloride - 20.0%). Manufacturer: Production and Scientific Enterprise "Ukrzoovetprompostach", Private Limited Company, Ukraine. According to GOST 12.1.00776, in terms of parameters of acute toxicity, the agent "Biodez" corresponds to the IV class – a low-hazardous substance, when applied to the skin and the III class – a moderately dangerous substance, when inhaled. The drug does not have cumulative, mutagenic, or carcinogenic properties.

Blanidas brand A. Manufacturer: Lysoform Medical LLC, Ukraine. The product's composition, the content of active and auxiliary substances, %: 1-Bromo-3-chloro-5,5-dimethyl hydantoin – 20-22% (the active substances), sodium tripolyphosphate, surfactants, corrosion inhibitors, table salt – up to 100.00. The content of active chlorine-bromine is at least 19.5%. Toxicity and safety of the product: according to the parameters of acute toxicity when injected into the stomach, when inhaled (in the form of vapor) and when applied to the skin, it belongs to the IV class of low-hazard substances. In its native form and the form of concentrated solutions (2.5%), it irritates the mucous membrane of the eyes and upper respiratory tract. In the recommended concentrations, it does not show skin-irritating properties or irritate the eyes.

Virkon-S. Manufacturer: Bayer. Composition: the active substance is potassium peroxymonosulfate, and auxiliary substances: sodium chloride, sulfamic acid, malic acid, sodium hexametaphosphate, sodium dodecylbenzene sulfonate, amaranth dye, flavouring with the smell of lemon. According to the level of toxicity, it belongs to moderately dangerous compounds (LD_{50} for white mice after oral administration is 3680 mg/kg of animal weight). In the recommended concentrations, it does not irritate the skin, slightly irritates mucous membranes, and does not cause sensitization.

Neochlor - (the active substance is sodium hypochlorite, and the product's initial content of active chlorine is from 7-9% (concentrate). The product's composition also includes detergent, anti-corrosion, stabilizing, antimicrobial, and flavouring additives). Manufacturer: Ukrainian Research and Production Center for Disinfection Problems CJSC (Ukraine). The agent "Neochlor" (a concentrate) belongs to the III class of dangerous (moderately dangerous substances); irritates the skin and mucous membranes; has weak cumulative properties; does not show a sensitizing effect; has no mutagenic properties.

Chlorination (dichloranthin, 5,5-dimethyl hydantoin is a chlorine-containing disinfectant of the third generation, active chlorine is not less than 13.5%); Manufacturer: "Farmakos" Scientific and Production Limited Liability Company (Ukraine). The product's composition, the content of active and auxiliary substances, and mass. %: 1,3-dichloro-5,5-dimethyl hydantoin (dichloranthin) – 21.5-23.5 (active ingredient); 5,5-dimethyl hydantoin – 12.5-16.5; dispersant – 9.0-12.5; anionic surfactants – 3.2-5.0; corrosion inhibitor up to 10.0; filler to 100.0. The mass fraction of active chlorine is at least 14.1%. Toxicity and safety of the product: chlorination belongs to moderately dangerous substances (hazard class 3) when ingested and inhaled into the body and low-hazard substances when applied to the skin (hazard class 4). In the conditions of inhalation action in the form of vapors, according to the degree of volatility, it belongs to low-hazardous substances. In dry form and concentrated solutions, it irritates the mucous membrane of the eyes and upper respiratory tract. In the concentrations recommended for cleaning and disinfection, it does not show skin-irritating properties and does not irritate the mucous membrane of the eyes. The product does not have skin resorptive and sensitizing, carcinogenic, mutagenic, or embryotoxic (according to the active substance) properties.

Phan (the active substance is didecyldimethylammonium chloride (at least 5%), auxiliary components (including surfactants and inorganic acid). Manufacturer: OU "BALTIACHEMI", Estonia. According to the parameters of acute toxicity when injected into the stomach, it belongs to class 3. In inhalation exposure and application to the skin, it belongs to the IV class of hazards (low-hazardous substances according to DSTU 12.1.007-76). It has no teratogenic or carcinogenic effect.

Clarified solution of perchloric lime. Chlorine lime is a mixture of hypochlorite, chloride, and calcium hydroxide. It belongs to the so-called mixed salts. Depending on the production method, chlorinated lime is produced in two grades: A and B. Moderately toxic. LD50 for rats – 850 mg/kg.

Animals, Plants and Biological Materials

Standardized cell cultures were obtained from the Cell Line Bank of the RE Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology, National Academy of Sciences of Ukraine.

Instruments

The research used disinfectants registered in Ukraine according to the established procedure that has different active substances belonging to different groups of chemical disinfectants and is allowed for use in Ukraine

Laboratory Methods

The research was carried out at the Central State Testing Laboratory of the State Production and Consumer Service in the Kyiv region and the city of Kyiv, the laboratory of industrial toxicology and occupational hygiene

of the Institute of Occupational Hygiene of the Academy of Medical Sciences of Ukraine, which the National Accreditation Agency of Ukraine accredits following DSTU requirements.

Cytotoxic properties were evaluated by three main tests, which took into account the viability of cells and their number – methyl tetrazolium test (MTT) **[26]**, staining cells with neutral red (NRP) **[27]** and the sulforhodamine B (SRB) test **[28].**

The use of in vitro toxicity tests is stipulated in Directive 2010/63/EU, which revises Directive 86/609/EEC on protecting animals used for experimental and other scientific purposes, adopted on September 22, 2010. The Directive is firmly based on the Three Rs principle to replace, reduce and improve the use of animals used for scientific purposes [**29**].

The studies were part of the research topic of the veterinary and sanitary examination department of the National University of Bioresources and Nature Management of Ukraine "Scientific assurance of the production of livestock products according to the Codex Alimentarius" (state registration number 0109U003215).

Description of the Experiment

Laboratory animals were not used.

Sample preparation: Cell cultures were stored in a frozen state. Cell cultures were cultured in vials. A new number of cells was thawed after two months of cultivation. Disinfectants were prepared before the test according to the producer's instructions. Distilled, deionized sterile water was used as solvent. Subsequently, to obtain the required concentration applied directly to the well, the working solution of disinfectants was added to the medium for cells so that the solvent in the first wells was not more than 2%.

Number of samples analyzed: 7 disinfectants in different concentrations were tested (10000, 5000, 2500, 1250, 625, 312,160, 80, 40, 20 mg/kg).

Number of repeated analyses: All measurements were performed 5 times.

Number of experiment replication: The number of replicates of each experiment to determine one value was 5 times.

Design of the experiment: They were cultured in complete nutrient medium DMEM (“SIGMA”, USA) containing 4 mmol/L of L-glutamine, 10% of fetal calf serum (“SIGMA”, USA), 40 μg/ml of gentamicin in a humidified atmosphere with 5% CO_2 at a temperature of 37 °C. The medium was changed every 2 days. Transplantation of cells was carried out with the help of Versen’s solution when the cells formed a continuous monolayer on the substrate (4-5th day of growth).

To study the sensitivity of cells to disinfectants, the cell suspension was placed on 96-well tablets at a concentration of 5×10^3-1×10^4 cells/well in 100 μl of nutrient medium. After 24 hours, a solution of the studied disinfectants at 20-100000 mg/kg was added to the cells and cultured at 37 °C in a humidified atmosphere for 24-48 hours.

Afterwards, changes in the metabolic state of the cells were assessed by:

1.a decrease in total mitochondrial dehydrogenase activity in the microculture tetrazolium test (MTT – photometric method) reflects inhibition of cellular respiration intensity. The tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is taken into cells reduced in a mitochondria-dependent reaction to yield a formazan product. The product accumulates within the cell, because it cannot pass through the plasma membrane. The product is liberated on solubilisation of the cells and can readily be detected and quantified by a simple colourimetric method. The cells' ability to reduce MTT indicates mitochondrial integrity and activity which, in turn, may be interpreted as a measure of viability and/or cell number **[26]**.

The activity of lysosomes, which easily capture and accumulate neutral red dye in living cells (neutral red test) **[27]**.

Protein synthesis, the amount of which in cells was determined by the sulforhodamine B (SRB) assay **[28]**. According to the methods, dyes were added to the wells of the plate, and incubated in the thermostat for a certain time, and the optical density of the good contents was determined using a Multiscan Microplate spectrometer (Sweden). Empty wells and wells with cells in which no xenobiotic was added were used as controls and controls with solvent, i.e. distilled deionized water (2%) in the cell culture medium.

Live cells were stained because (depending on the method) formazan dye accumulates in the cell, the Neutral Red dye accumulates in the lysosomes, and Sulforhodamine B dye binds to the protein components of the cell.

After 24-48 hours, the studied compounds were added to the growth medium in different concentrations, and the cells were incubated under standard conditions for 24 hours, after which the cells were stained with one of the methods (with tetrazolium blue, with sulforhodamine B, and with neutral red).

Statistical Analysis

Statistical differences between experimental and control results were tested by one-way analysis of variance (ANOVA) and the student-Newman-Keuls test. Values of $p < 0.05$ were considered statistically significant. Statistical processing was performed in Microsoft Excel 2016 values were estimated using mean and standard deviations and subsequently evaluated in the statistical program XL Stat. In hypothesis testing, if the *p*-value is lower than a significant level, in the case of XL Stat software by Addinsoft (version 2019.3.2), it is 0.05, the null hypothesis was rejected and alternative hypothesis was confirmed.

RESULTS AND DISCUSSION

Determination of the toxicity of disinfectants in vitro was carried out on HT-29 and HEK 293 cell cultures. Cell cultures have a stable, homogeneous cell morphology, are well attached to the surface of the bottom of the wells and are also characterized by good growth potential. We also considered that the digestive organs and kidneys are of particular importance in the processes of xenobiotic excretion. In scientific works **[29]**, **[30]**, **[31]**, similar series of experimental studies are described, which were conducted in a later period and were focused on the study of the metabolism of various substances. As it is known, the reason for the toxic effect of xenobiotics on living systems is their ability to disrupt the course of basic biochemical processes (for example, protein biosynthesis, respiration, energy exchange, and substance metabolism).

It shall be noted that the content of disinfectants, detergents, and disinfectants in food products is not allowed by both national regulations and EU legislation (Ministry Order 2646:2019; DSTU 3662:2018 **[32]**. Directive (EU) No. 853 (2004), but their residual amounts in food products can cause negative effects on human health **[33]**. The scientific works **[34-37]** described a series of experimental studies, the purpose of which was to determine the residual content of disinfectants and detergents and disinfectants in food products using various methods and techniques.

The results obtained by us regarding the determination of the cytotoxic effect of disinfectants on the HT-29 cell culture (intestinal epithelium model) (Table 1) show that a clarified solution of perchloric lime caused the destruction of the monolayer and close to 100% cell death in concentrations that correspond to the concentrations of the working solutions of disinfectants and detergents (5000-10000 µl/l) solutions of Biodez, Blanidas, Vircon-S, neochlor, (Figure 1). A series of similar experimental studies were described in scientific works **[38]**, **[39]**, **[40]**, but the researchers used 293-T human kidney (embryonic) with different concentrations that correspond to the concentrations of working solutions of disinfectants and detergents and disinfectants (4500-9000 µl/l)

Biodez demonstrates significant cytotoxicity for HT-29 cell culture. Even at a concentration of 20 µl/l, cell survival is only 66.38 ±2.44% of cells ($p < 0.001$). It has almost the same cytotoxic effect on HT-29 and Phan cells. According to our research, 59.83 ±7.98% of the cells of this line survive at a concentration of 20 µl/l. Blanidas, Neochlor at a concentration of 20 µl/l do not cause cell death (the cell culture monolayer does not differ from the control) (Figure 2).

Table 1 Cytotoxic effect of disinfectants on cells of the HT-29-line, M ±m, n = 18.

	Cell viability indicators, %									
Concentration, mg/kg	**10000**	**5000**	**2500**	**1250**	**625**	**312**	**160**	**80**	**40**	**20**
Biodez	–	4.21 ±0.5*	8.44 ±2.11*	23.47 ±2.65*	24.49 ±5.20*	35.71 ±7.12*	37.90 ±3.25*	44.8 ±2.44*	**55.71 ±1.22***	66.28 ±2.44*
Blanidas	8.41 ±1.35*	26.17 ±2.03*	26.17 ±2.03*	24.30 ±5.09*	28.04 ±5.09*	42.99 ±1.02*	**65.33 ±5.09****	99.07 ±1.02	-	-
Virkon-S	2.88 ±1.75*	4.07 ±2.13*	5.75 ±3.32*	18.67 ±3.03*	32.83 ±9.57*	**44.69 ±6.55***	73.1 ±9.34	82.68 ±3.62	71.42 ±5.67$	-
Neochlor	2.92 ±0.80*	8.84 ±2.36*	14.8 ±2.55*	25.64 ±5.18*	**49.45 ±3.89***	54.95 ±2.59*	75.09 ±12.2	76.92 ±18.1	95.24 ±2.56	100.73 ±9.1
Chlorantoin	7.92 ±0.80*	15.84 ±1.61*	12.45 ±0.80*	33.94 ±1.61*	39.60 ±7.23*	**47.52 ±4.82***	64.49 ±7.23**	67.89 ±9.64$	76.94 ±8.04	85.99 ±6.43
Chlorinated lime	10.79 ±3.0*	7.19 ±3.95*	10.27 ±3.41*	13.36 ±3.67*	17.47 ±5.88*	47.26 ±6.89*	**52.4 ±7.17***	64.73 ±3.67*	80.11 ±1.24$	95.05 ±2.99
Phan	6.11 ±1.0*	7.33 ±1.20*	9.16 ±0.86*	10.01 ±1.11*	20.51 ±1.79*	20.76 ±1.0*	34.19 ±1.99*	39.07 ±9.51*	46.4 ±5.51*	**59.83 ±7.98****

Note: **$** – $p < 0.05$; ** – $p < 0.01$; * – $p < 0.001$ – relative to the control.

The death of single cells and a slight decrease in marker functions in the culture of HT-29 cells were detected at a concentration of 20 µl/l of chlorination and a clarified solution of perchloric lime.

Thus, the IC_{50} (average inhibitory concentration is the concentration of the drug that suppresses a certain cellular function by 50%) of disinfectants based on the effect on the culture of intestinal origin (HT-29) is Biodez – 60.93 ±9.81 µl/l, Blanidas – 264.30 ±25.12 µl/l, Virkon-S – 283.59 ±31.20 µl/l, Neochlor –593.70 ±33.86 µl/l, Phan – 69.28 ±8.4 µl /l, Chlorination – 289.79 ±30.85 µl/l, Chlorinated lime – 117.35 ±9.44 µl/l. The authors of the scientific works **[41]**, **[42]**, **[43]**, conducted a similar series of experimental studies. However, the effect on the cell line of kidney origin (NEC 293) was: Biodez - 123.65 ±19.56 µl/l, Blanidas – 314.20 ±16.54 µl/l, Virkon-C – 382.48 ±24.87 µl/l, Neochlor – 153.94 ±26.45 µl/l, Fan – 34.13 ±5.7 µl/l, Chlorination – 306.76 ±23.66 µl/l, in our opinion, this is due to non-compliance with the temperature regime during the experiments.

Figure 1 Cells of the NT-29 line, after exposure to disinfectants at a concentration of 2500 µl/l (staining with neutral red).

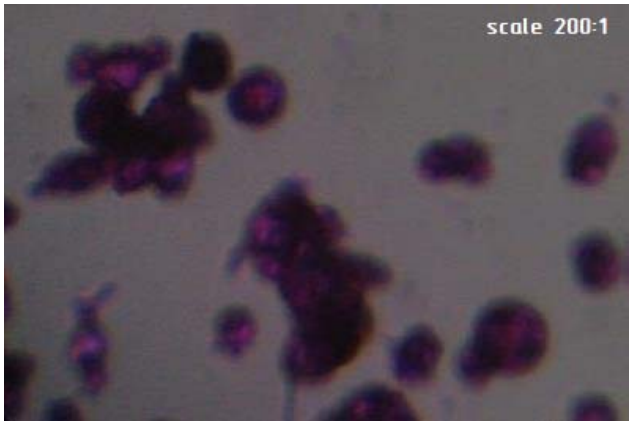

Figure 2 Accumulation of sulforhodamine by HT-29 culture cells (Neochlor – 40 µl/l) (test with SR).

We used the HEK 293 (Human Embryonic Kidney) cell line as a kidney model to determine the organ-specific toxicity of the studied disinfectants (Figure 3).

The obtained results (Table 2) indicate the high cytotoxicity of disinfectants and detergents on the cells of this line. At concentrations of 5,000-10,000 µl/l, all the studied agents cause the detachment of cells from the bottom of the wells and their death. Virkon-S, Blanidas, Neochlor, and Chlorination are the least toxic during the study of in vitro toxicity of the disinfectants and detergents studied.

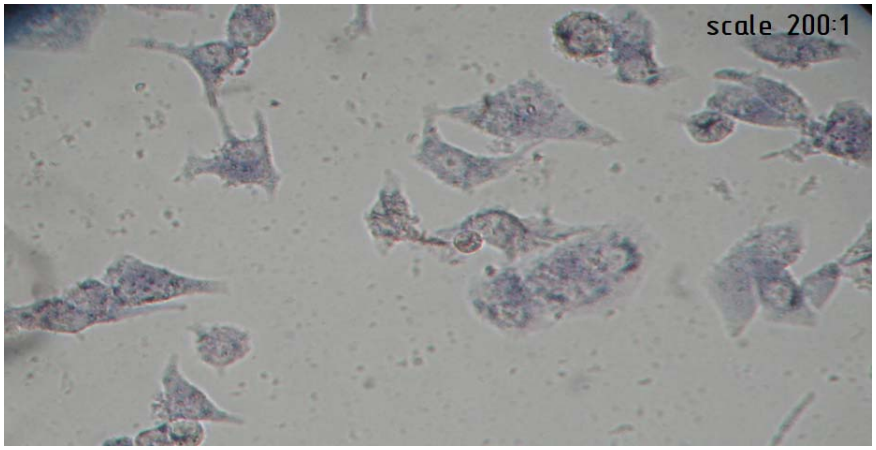

Figure 3 Monolayer of HEK 293 cells (control) ((NRP method)).

Table 2 Cytotoxic effect of disinfectants on cells of the HEK 293-line, M ±m, n = 18 %.

	Cell viability indicators, %									
Concentration, mg/kg	10000	5000	2500	1250	625	312	160	80	40	20
Biodez	0	0	6.90 ±1.48*	14.06 ±4.35*	29.19 ±7.38*	37.15 ±3.69*	**61.03 ±7.38****	79.60 ±33.21	92.87 ±14.76	84.91 ±14.76
Blanidas	0	2.26 ±0.39*	22.60 ±3.91*	44.29 ±3.13*	45.2 ±3.91*	**58.76 ±2.83***	63.28 ±3.91*	70.06 ±1.96**	63.28 ±9.57$	72.32 ±3.91**
Virkon-S	2.36 ±0.18*	11.67 ±0.98*	23.88 ±12.78*	31.84 ±3.21*	42.45 ±5.38*	**53.07 ±4.47***	68.99 ±5.38**	106.14 ±6.34	-	-
Neochlor	0.24 ±0.03*	2.07 ±0.5*	3.62 ±0.98*	16.27 ±2.21*	22.6 ±1.84*	**54.24 ±11.07***	58.76 ±3.69*	63.28 ±3.29*	70.96 ±3.15**	72.77 ±3.52**
Phan	5.42 ±0.68*	14.92 ±1.17*	20.34 ±3.39*	23.73 ±1.69*	27.12 ±6.78*	33.90 ±3.39*	36.61 ±9.02*	43.39 ±2.35*	**54.24 ±6.78***	61.02 ±3.39*
Chlorantoin	7.55 ±0.9*	11.2 ±1.80*	14.59 ±1.85*	18.57 ±3.69*	23.88 ±6.39*	**59.7 ±3.20***	68.19 ±2.58**	75.89 ±3.22$	90.22 ±3.69	92.87 ±3.69

Note: **$** – p <0.05; ** – p <0.01; * – p <0.001 – relative to the control.

Phan, Neochlor, and Blanidas exhibited the most significant cytotoxic effect on the HEK 293 line cells. These disinfectants and detergents at a concentration of 20 µl/l of the growth medium cause about 30-40% cell death.

The IC_{50} of disinfectants based on the effect on the cell line of kidney origin (HEK 293) is Biodez – 173.65 ±19.56 µl/l, Blanidas – 514.20 ±16.54 µl/l, Virkon-S – 402.48 ±24.87 µl/l, Neochlor – 353.94 ±26.45 µl/l, Phan – 44.13 ±5.7 µl/l, Chlorination – 396.76 ±23.66 µl/l (Figure 4). The authors of the scientific works **[44]**, **[45]**, **[46]**, **[47]**, conducted a similar series of experimental studies, but the effect on the cell line of kidney origin (NEC 293) was: origin (HEK 293) is: Biodez – 153.65 ±19.56 µl/l, Blanidas – 414.20 ±16.54 µl/l, Virkon-S – 302.48 ±21.57 µl/l, Neochlor – 323.91 ±22.05 µl/l, Phan – 34.11 ±3.7 µl/l, Chlorination – 396.21 ±20.06 µl/l, in our opinion, such a difference may be related to non-compliance with both the temperature regime during experiments and the non-compliance with time regimes.

Biodez, (the active ingredient is polyhexamethylene guanidine hydrochloride) is more toxic in low concentrations for cells of intestinal origin and somewhat less toxic for cells of renal origin. Blanidas, a chlorine disinfectant, exhibits greater cytotoxicity on cells of the HT-29 line and somewhat less on the HEK 293 cell line. Virkon-S is characterized by high toxicity for the culture of HEK 293, and HT-29 cells. Neochlor (a chlorine-containing disinfectant) is most toxic at low concentrations for cells of kidney origin and somewhat less toxic for cells of intestinal origin. A detergent-disinfectant phan showed high cytotoxicity on HEK 293 and HT-29 cells. Chlorination (chlorinated disinfectant of the third generation) is highly toxic for cells of renal and intestinal origin. The clarified solution of perchloric lime has a significant toxic effect on all the studied cell lines.

The cytotoxic effect of disinfectants was characterized by the phenomena of vacuolization, balloon-like dystrophy, and toxigenic cell lysis (Figure 5). It is worth noting that almost all studied disinfectants and detergents showed high toxicity in vitro on cell cultures of renal and intestinal origin.

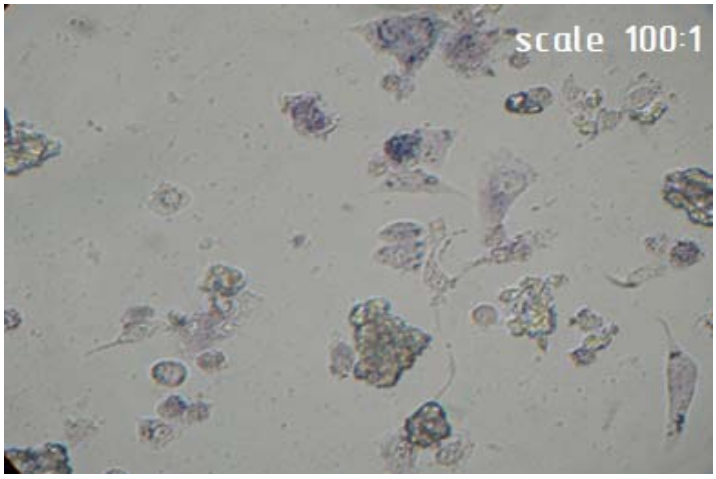

Figure 4 The state of the HEK 293 cell culture monolayer after the introduction of disinfectants into the growth medium at a concentration close to IC50 (MTT method) (×100).

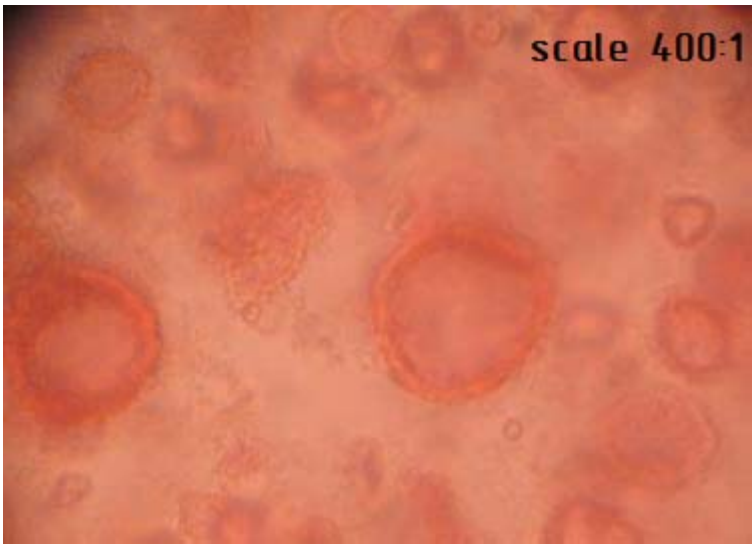

Figure 5 Cytoplasm lysis of HEK 293 cells after the introduction of disinfectants (NRP method).

In further studies, it may be worth evaluating their effect also on other cell cultures, such as those of lung origin and skin.

The average toxic dose (IC50), at which 50% of cells survived and remained attached to the surface, is Biodez – 110.77 ±23.6 µl/l, Blanidas – 594.82 ±36.78 µl/l, Virkon-S – 545.78 ±31.36 µl/l, Neochlor – 603.7 ±49.55 µl/l, Phan – 603.7 ±36.8 µl/l, Chlorination – 393.44 ±54.11 µl/l, clarified solution of perchloric lime – 121.98 ±9.87 µl/l.

It is known that any drug toxicity is determined in model systems, which are both in vivo models using laboratory animals and in vitro based on cell cultures. In both cases, interpreting the results to a greater or lesser extent has the character of possible approximation.

In vitro methods make it possible to explain biological phenomena that, due to the interaction of various factors, are difficult to study in experiments on animals; they contribute to the deepening of understanding by highlighting molecular and cellular mechanisms **[48]**, **[49]**, **[50]**.

To prove the possibility of studying the toxicity of disinfectants at the stage of preliminary toxicological assessment and determining the target organs of toxic effects by in vitro methods, we compared the in vivo toxicity indicators for laboratory animals (according to the literature) and the in vitro cytotoxicity results obtained by us (Table 3). In addition, it shall be emphasized that the determination of cytotoxicity cannot provide complete data on the toxic effect on the entire body.

Based on the comparison of literature data on the toxicity of disinfectants obtained on laboratory animals with our data on cytotoxicity, there can be made a generalization: based on the results of the cytotoxic effect on human cell cultures, preliminary conclusions can be drawn regarding the toxicity of the substance at the stage of screening chemicals for certain purposes, preliminary hygienic regulation, etc. and establish target organs of toxic influence.

Table 3 Toxicity of the studied disinfectants (active substances) in vivo and in vitro.

	Animal	Administration	LD_{50} mg/kg	IC_{50}		$IC_{50\ avg}$
				HEK 293	HT-29	
Biodez	white rats	orally	600 [30]	173.65 ±19.56	60.93 ±9.81	**117.29 ±14.69**
Blanidas	white rats	orally	>2000	514.20 ±16.54	264.30 ±25.12	**389.25 ±20.83**
Virkon-S	white rats white mice	orally	4120 3680	402.48 ±4.87	283.59 ±31.20	**343.04 ±28.04**
Neochlor	rats	orally	2540	353.94 ±26.45	593.70 ±33.86	**473.82 ±30.16**
Phan	white rats	orally	1470	44.13 ±5.7	69.28 ±8.4	**56.71 ±7.05**
Chlorination	Rats (3-colorant in-. 5-dimethyl hydantoin)	orally	542	396.76 ±23.66	289.79 ±30.85	**343.28 ±27.26**
Chlorine lime	Ca $(ClO)_2$. $CaCl_2$ and Ca $(OH)_2$ mixture	orally	-	-	117.35 ±9.44	117.35 ±9.44

Note: * – for a clearer comparison of LD50 and IC50 indicators, the digital values of the latter were expressed as mg/l or mg/dm^3.

However, it shall be noted that in the case of finding the toxicity class of substances, it is impossible to be guided only by the data obtained from the cytotoxic effect. It is necessary, as during toxicological studies on laboratory animals, to take into account the results of other studies, including mutagenic effects, etc.

When comparing the data obtained by us in the experiments and the data of other scientists regarding the toxicity of the studied substances, certain discrepancies can be noted. Thus, the average toxic doses for cell cultures (IC50) of all studied disinfectants are lower than the LD50 obtained in animals.

In addition, data from various scientific sources on the toxicity of disinfectants and their active substances obtained using animals differ significantly depending on the drug's administration method, animal species, duration of exposure, etc. **[51]**, **[52]**. Data on the toxicity of disinfectants for laboratory animals were taken from literature sources.

The results of our research allow us to state that the cytotoxic effect of disinfectants of different chemical natures has a stereotypical character that does not significantly depend on the agent's chemical structure and the cell line used for research (within the class of mammals).

The existing method of determining the residual quantities of disinfectants and detergents on the technological equipment of enterprises for the production, processing, and transportation of food products is based on determining the pH of the surface using universal indicator paper strips with a range of values from 2 to 11. The change in the indicator's colour, detergents, and disinfectants determines the presence of residual disinfectants. But, since a significant part of modern detergents and disinfectants in working concentrations has a pH close to the pH of drinking water (6.0–9.0), this method is not effective enough, and therefore it is impossible to establish the presence of disinfectants and detergents on technological equipment reliably **[53]**.

There are methods for determining disinfectants and detergents in the last portion of washing water. But these methods are specific for each disinfectant, active substance, or chemical class **[54]**, **[55]**.

To assess the risk associated with the use of disinfectants and guarantee the safety of human and animal health, it is required to have sensitive methods for determining their insignificant concentrations in various environments and control the content of residual amounts of toxicants in environmental objects, feed, and food products.

The study's results can be useful during the preliminary testing of disinfectants and the development of a method for determining the residual amounts of disinfectants and detergents in biological objects, particularly milk, and washings from the surface technological equipment, using the determination of cytotoxicity of milk samples.

CONCLUSION

The average toxic dose (IC50), at which 50% of cells survived and remained attached to the surface, is Biodez – 110.77 ±23.6 µl/l, Blanidas – 594.82 ±36.78 µl/l, Virkon-S – 545.78 ±31.36 µl/l, Neochlor – 603.7 ±49.55 µl/l, Phan – 603.7 ±36.8 µl/l, Chlorination – 393.44 ±54.11 µl/l, clarified solution of perchloric lime – 121.98 ±9.87 µl/l, which is two to three times higher than the LD_{50} obtained in animals.

Vacuolization, balloon-like dystrophy and toxigenic cell lysis characterized the cytotoxic effect of disinfectants. It is worth noting that almost all studied disinfectants and detergents showed high toxicity in vitro on cell cultures of renal and intestinal origin.

Studying the cytotoxicity of disinfectants and detergents in vitro using human cell cultures of different histogenesis, which are used during the technological operations of food production, can significantly reduce the number of animals for establishing LD_{50} during the registration procedure of new agents. In particular, based on the results of the cytotoxic effect on human cell cultures, preliminary conclusions can be made regarding the toxicity of the substance at the stage of chemical screening, preliminary hygienic standardization, etc., and there can be identified the target organs of the toxic effect.

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