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ORIGINAL ARTICLE

Comparative *in vitro* cytotoxicity of active disinfectant substances and a commercially available surface disinfectant on human respiratory epithelial cells

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ABSTRACT
Disinfection of hands and surfaces is a key element in preventing the spread of infections. In this study, the cytotoxic effect of chemical substances used in conventional disinfectants was assessed: n-propyl alcohol (1P), isopropyl alcohol (2P), ethyl alcohol (EtOH), phenoxyethanol (FenEtOH), glutaraldehyde (GA), didecyldimethylammonium chloride (DDAC), sodium diisocyanurate (NaDCC), potassium peroxymonosulfate (KPMS) and the ready-to-use commercially available mixture for surface disinfection Mikrozid AK liquid (MAF). The assessment was performed on two cell lines derived from the human respiratory system: BEAS-2B and A549. To assess the cytotoxic effect of the tested compounds/mixture, the MTT and NRU assays were used. Based on the obtained results, DDAC and GA were found to be the most cytotoxic, while NaDCC and KPMS exhibited slightly lower levels of cytotoxicity. The alcohols: 1P, 2P and EtOH were characterized by lower toxicity on cells of both lines. The commercially available MAF mixture was the least toxic to A549 cells. Moreover, 2P, GA and EtOH exhibited a stronger cytotoxic effect on the metabolic activity of cells assessed by the MTT in both tested respiratory system-derived cell lines, compared to their effect on cell membrane integrity, which was evaluated using the NRU test. The obtained results also indicate that BEAS-2B cells are significantly more sensitive to the cytotoxic effects of the tested compounds than A549 cells, a difference that was particularly evident upon exposure to 1P, GA, DDAC, NaDCC and MAF in both tests.

KEY WORDS: disinfectants; active substances; cytotoxicity; respiratory system cells; ***in vitro***

Introduction

In recent years, efforts to combat and prevent the spread of infections have become a major global concern due to the SARS-CoV-2 coronavirus. One of the key elements in counteracting the continued spread of the COVID-19 pandemic was prevention. In addition to vaccination, preventive measures included disinfection, maintaining physical distance, avoiding large gatherings, and protecting against droplet transmission by covering the nose and mouth (*e.g.*, wearing masks). Considerable attention was given to disinfecting surfaces, such as door handles and other objects frequently touched by potentially infected individuals. Although the effectiveness of disinfection is a key condition, careful consideration must also be given to minimizing the toxic impact of disinfectants on human health and the natural environment. This toxic effect is highly probable, considering the fact that the mechanism of antimicrobial action of all biocides is based precisely on their toxic effect on microbial cells. It is strictly dependent on their chemical structure and physicochemical properties (*i.e.* molecular size, solubility of the substance, ability to dissociate and ionize) and biochemical properties (*i.e.* type of chemical bonds and functional groups, oxidizing and alkylating properties). After the initial molecules adsorption to the surface of the bacterial cell, the substance penetrates and accumulates inside the microorganism's cell to a level sufficient to obtain a toxic effect. The penetration of biocides depends on both their physicochemical properties and the structure of cellular barriers, whose primary function is to protect against the toxic effects of chemical substances (Książczyk *et al.*, 2015). The most commonly used active substances in chemical disinfectants include: alcohols (mainly

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ethyl, isopropyl and n-propyl alcohol), chlorine and its compounds, formaldehyde, glutaraldehyde, hydrogen peroxide, iodophors, peracetic acid, persulfates, phenols and quaternary ammonium compounds (Artasensi *et al.*, 2021; Cota *et al.*, 2023). Most of these substances can irritate the mucous membranes of the eyes, upper respiratory tract and skin, especially when used in large quantities or in enclosed spaces. They may also exhibit allergenic properties (Brans & Skudlik, 2025; Ibrahim *et al.*, 2025) and release toxic gases. Occupational exposure to disinfectants has been associated with cardiovascular diseases among nurses (Wang *et al.*, 2025). It is important to remember that although inhalation is the primary route of exposure, these compounds, *e.g.* glutaraldehyde, can also be absorbed dermally and orally.

To maintain the balance between maximal disinfectant efficacy and minimal toxicity for both human health and the environment, cytotoxicity assessment of the chemical substances found in disinfection preparations is essential. It is worth noting that the relationship between biocidal activity and toxicity of the disinfectant may vary depending on the type of pathogen (Wu *et al.*, 2012).

An important aspect of choosing the right disinfectant is maintaining the most favourable relationship between their antibacterial and cytotoxic effects. Iwasawa *et al.* (2011) conducted tests of bactericidal and cytotoxic effects for samples containing glutaraldehyde (GA) at a concentration of 2.25% and 3.5%, orthophthalaldehyde (OPA) at a concentration of 0.55% and for glutaraldehyde alone. The obtained results indicated that all tested disinfectants showed sufficient bactericidal effects on *Mycobacterium terrae*, while the OPA product was less toxic than products with GA and GA alone for the four tested cell lines (rabbit corneal epithelial cells SIRC; human conjunctival Chang cells; rabbit skin cell line FRSK and HeLa S3 cells derived from human cervical cancer), which may be due to the lower concentration of the active ingredient (OPA) in the product. The most sensitive cell line to glutaraldehyde-based disinfectants was the Chang cell line, while all lines showed similar sensitivity to OPA.

The study results indicate the need to assess the toxicity of disinfectants in many exposure variants, starting with the route and time of exposure, concentration, and ending with the selection of various cell lines. Disinfectants are effective in preventing viral infections, however according to Simitcioglu *et al.* (2021), there is still a lack of clear evidence regarding their potential destructive effects on healthy cells of individuals using them. The authors conducted an evaluation of the cytotoxic activity of hand disinfectants on healthy human umbilical vein endothelial cells (HUVEC) using the *in vitro* method tetrazolium salt reduction test determining the metabolic activity of cells (MTT), which allowed them to conclude that disinfectants containing ethyl alcohol and ammonium compounds, isopropyl alcohol, and chlorhexidine gluconate have cytotoxic effects on healthy human cells.

By assessing the cytotoxic effect in *in vitro* studies using human cell cultures: HT-29 (colon adenocarcinoma epithelial cells) and HEK 293 (human embryonic kidney cells), Iakubchak *et al.* (2023) found that the average toxic doses for cell cultures are lower than the average lethal doses determined for rats and mice after intragastric administration. Based on MTT and neutral red uptake (NRU) tests, IC_{50} values were determined for seven commercially available disinfectants containing various active substances (including quaternary ammonium compounds, inorganic hypochlorous acid salts), which allowed them to conclude that almost all tested agents showed high *in vitro* toxicity on cell cultures of renal and intestinal origin. Similarly, Zhang *et al.* (2023) noted the strong cytotoxic effects of povidone-iodine, chlorhexidine acetate, and polyhexamethylene biguanide (PHMB) on fibroblasts (HF cells) and keratinocytes (HaCaT). The povidone-iodine was found to be the most cytotoxic, even at low concentrations, and the need to pay special attention to the time of use and concentration of disinfectants in clinical settings was emphasized. Sagripanti and Bonifacino (2000), based on studies of the cytotoxic effect of disinfectants, conducted *in vitro* using the MTT test on cell lines of various origins and proposed classifying liquid disinfectants based on their toxicity as mild (including phenol, hydrogen peroxide and formaldehyde), moderately toxic (sodium hypochlorite) and highly toxic (including glutaraldehyde, copper(II) ascorbate and peracetic acid). Neuman *et al.* (1998) found that 24-hour exposure of Hep G2 cells to ethanol at a concentration of 80 mmol/l increased the expression of cytokines: IL-1a (42%), IL-6 (26%) and TNF-α (52%) compared to the control. Glutathione (GSH) was also depleted by 55% (both cytosolic and mitochondrial). Thomes *et al.* (2023) found that ethanol causes the accumulation of protein aggregates by inducing proteasome inhibition in VL-17A hepatoma cells ,which were derived from the human liver cancer cell line HepG2 and which, unlike HepG2 cells, metabolize ethanol because, as a result of transfection, they stably express enzymes involved in the oxidative metabolism of ethanol: alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1).

Quaternary ammonium compounds (QACs) are widely used to kill viruses, bacteria, algae and other pathogenic microbes, providing a substantial public health benefit (Osimitz & Droege, 2025). But some of these compounds can cause lung inflammation and fibrosis, albeit the mechanism of action is not clear. Kwon *et al.* (2014) based on the results of didecyldimethylammonium chloride (DDAC) toxicity studies at the cellular level suggested that exposure to even low concentrations of DDAC can inhibit cell growth and cause oxidative stress in lung epithelial cells. DDAC-induced cell damage was associated with mitochondrial and lysosomal damage with the release of lactate dehydrogenase (LDH). In addition, DDAC caused an increase in the level of reactive oxygen species (ROS) in cells and a decrease in GSH activity. It is worth noting that the cytotoxic effects of disinfectants on cells can be observed at concentrations much lower than their bactericidal effect. In the studies conducted by Hidalgo *et al.* (2002), the cytotoxic effect of sodium hypochlorite (NaOCl) on human skin fibroblasts *in vitro*

was found, which consisted in the depletion of cellular ATP, already at concentrations of 0.00005%, while the bactericidal effect of the compound was observed only at a concentration of 0.5% (at concentrations of 0.25–0.025% the compound showed partial bactericidal effect). NaOCl at concentrations higher than 0.05% caused a decrease in cell viability to zero values.

Materials and methods

The following substances and mixture (detailed in Table 1) were tested on respiratory cells: propan-1-ol (1P); 2-propanol (2P); glutaraldehyde (GA); 2-phenoxyethanol (FenEtOH); ethanol (EtOH); N-decyl-N,N-dimethyl-1-decanammonium chloride (DDAC), 3,5-dichloro-2-hydroxy-4,6-s-triazinedione sodium salt (NaDCC); potassium peroxymonosulfate (KPMS) and MIKROZID AF liquid (MAF).

The substances tested were purchased from Merck Life Science (formerly Sigma-Aldrich Chemical Company, St. Louis, Mo USA), Pol-Aura Sp. z o.o., Avantor Performance Materials Poland S.A. (formerly POCH S.A.). Mikrozid AF liquid came from the MEDISEPT Sp. z o.o. store (Dezynfekcja.pl). The studies were performed on cells of the immortalized line of human bronchial epithelium BEAS-2B (ATCC® CRL-9609™) and A549 cell line (ATCC® CCL-185™) derived from epithelial-like cells of human non-small cell lung cancer, which were purchased from LGC Standards Sp. z o.o. from the American collection of ATCC (American Typical Culture Collection).

The following cell culture media were used for cell culture: DMEM (catalog no. 31885) and LHC-9 (catalog no. 12680-013); Foetal Bovine Serum (catalog no. 10084-150), Antibiotic – Antimycotic (penicillin G sodium, streptomycin sulfate, amphotericin B and fungizone) (catalog no. 15240-062) from Gibco BRL (Thermo Fisher Scientific, formerly Life Technologies Ltd. Paisley, Scotland) and trypsin solution (0.25%) and EDTA (catalog no. T-4049) from Merck. The following tests were used for testing: 0.4% trypan blue solution (catalog no. T-8154); NRU (catalog no. N-2889); MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] (cat. no. M-5655); dimethyl sulfoxide (DMSO) (cat. no. D-5879); Hanks buffer (Hank's Balanced Salt Solution) (cat. no. H-8264); Dulbeco's Phosphate Buffered Saline (cat. no. D-8537) from Merck.

Cell culture

Cell culture was carried out in culture vessels with a culture surface of 25 cm^2 (NUNC), in the form of a single layer at the bottom of the vessel (monolayer). A549 cells were cultured in DMEM nutrient medium, which contained 10% fetal bovine serum and antibiotics (Penicillin, Streptomycin, Amphotericin, Neomycin) (1ml/100 ml medium), while BEAS-2B cells were cultured in LHC-9 medium.

Cells were incubated in sterile conditions, in an incubator with access to 5% CO_2, at a constant temperature of 37 °C and humidity of 95%. The growth of the culture was assessed under a microscope. Every few days (depending on the observed growth), the nutrient

Table 1. Tested substances/mixture.

EC-Name	IUPAC Name	Abbrev.	CAS-No. EC-No.	Purity	Supplier (cat. no.)
Propan-1-ol	Propan-1-ol	1P	71-23-8 200-746-9	≥ 99.90%	Merck Life Science Sp.z.o.o (Sigma Aldrich 34871)
Propan-2-ol	Propan-2-ol	2P	67-63-0 200-661-7	≥ 99.85 %	Merck Life Science Sp.z.o.o. (Sigma Aldrich 34863)
Ethanol	Ethanol	EtOH	64-17-5 200-578-6	99,8% CZDA	Avantor Performance Materials Poland S.A. (formerly POCH S.A. 396480111)
2-Phenoxyethanol	2-Phenoxy ethanol	FenEtOH	122-99-6 204-589-7	n.a. (Sigma Aldrich quality level 200)	Merck Life Science Sp.z.o.o. (Sigma Aldrich 77699)
Glutaraldehyde	Pentanedial	GA	111-30-8 203-856-5	n.a. (Sigma Aldrich quality level 200)	Merck Life Science Sp.z.o.o (Sigma Aldrich 340855)
Didecyldimethylammonium chloride	N,N-Didecyl-N,N-dimethylammonium chloride	DDAC	7173-51-5 230-525-2	n.a.	Pol-Aura Sp. z o.o (PA-03-7231-K)
Troclosene sodium	1,3,5-Triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt	NaDCC	2893-78-9 220-767-7	n.a. (Sigma Aldrich quality level 200)	Merck Life Science Sp.z.o.o. (Sigma Aldrich 218928)
Pentapotassium bis(peroxymonosulphate) bis(sulphate)	Pentapotassium bis(peroxymonosulphate) bis(sulphate)	KPMS	70693-62-8 274-778-7	n.a. (Sigma Aldrich quality level 200)	Merck Life Science (Sigma Aldrich 228036)
Mikrozid® AF liquid	–	MAF	Composition: 100 g solution contains the following active substances: 24,75 g Ethanol (94 %), 35 g Propan-1-ol. Labeling according to Regulation (EC) No. 648/2004: perfumes		MEDISEPT Sp. z o.o. (Dezynfekcja.pl) (producer: Schülke & Mayr GmbH)

n.a. – not available

medium was replaced to provide the cells with access to growth factors and to remove metabolic products. A cell suspension was prepared before starting the studies. For this purpose, cells were separated using trypsin (0.25% trypsin solution with EDTA), then suspended in fresh culture medium and subjected to centrifugation (1200×g for 5 min) to remove cells damaged by trypsinization. Before starting the actual experiment, the obtained suspension was assessed for cell survival using the trypan blue uptake test. The cells were placed in a Bürker chamber and live (unstained) and dead/damaged (blue-stained) cells were counted. This assessment was performed using an inverted light microscope (Nikon TMS-F, Japan) using 100× magnification.

Exposure of cells to tested substances and preparation

Stock solutions of tested substances/mixtures at w/v or v/v concentrations were prepared immediately before performing cytotoxicity tests. Stock solutions were prepared by dissolving the tested solid compounds in water, and then solutions of each xenobiotic were prepared at six concentrations within the range of effective concentrations in the culture medium. The cytotoxicity test was performed in triplicate.

Cells were suspended in culture medium at a concentration of 1×10^5 live cells/ml and then placed in a 96-well microplate with 100 µl of suspension in each well. After 24 h of incubation (temp. 37 °C; 5% CO_2; 95% humidity), during which the cells stuck to the bottom of the microplate, the medium above the cells was aspirated and 100 µl of the solution of the tested substance/mixture of a specified concentration was added to each well of the microplate. Each sample was applied in 9 replicates. The control consisted of cells incubated in a nutrient medium devoid of xenobiotics. The microplates were placed in a CO_2 incubator for 24 h, after which the cytotoxicity of the tested compounds was determined.

Methods of assessing toxic effects

The following methods were used to assess the toxic effects of the tested compounds on cells *in vitro*:

- MTT test – MTT tetrazolium salt reduction test determining the metabolic activity of cells;
- NRU Assay assessing the integrity of cell membranes,

which are among the most commonly used assays, generating significant data within a short period of time. They are relatively inexpensive and highly sensitive (Figueiró *et al.*, 2016).

Assessment of cell metabolic activity based on the MTT tetrazolium salt reduction test

The MTT test, conducted in accordance with INVITTOX protocol no. 17 (1990) assesses cellular metabolic activity based on the ability of cells to absorb the yellow tetrazolium salt (MTT) and reduce it, primarily in the mitochondria, with the participation of succinate dehydrogenase to a formazan compound with a purple-navy blue colour.

The cells are first exposed to a series of different concentrations of the test substance/mixture and then incubated in a solution of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. After the incubation of the cells with the test compound/mixture, the solution of the test substance/mixture was removed from above the cells and 100 µl of MTT solution in Hanks buffer (0.5 mg/ml) was added to each well of the microplate. The cells were then incubated for 3 hours, after which the dye was removed from above the cells and the cell membranes were destroyed using DMSO (100 µl) and the precipitated formazan was dissolved. The concentration of the dye in the test and control samples was determined colorimetrically on a SYNERGY 2 reader at a wavelength of 570/630 nm. In order to thoroughly dissolve the dye in DMSO, the plates were shaken for 3 min. immediately before the measurement. Based on the obtained results, the concentration of the tested substance/mixture causing a decrease in the metabolic activity of the cells by 50% compared to the control (IC_{50}) was determined.

Assessment of cell membrane integrity based on the neutral red absorption test

The principle of the NRU test (INVITTOX protocol no. 64, 1992) is based on the ability of living, undamaged cells to absorb the dye – neutral red (3-amino-7-dimethyl-amino-2-methylphenazine hydrochloride), which accumulates in lysosomes.

After the incubation of cells with the tested compound/mixture (the cell exposure time selected based on the literature review was 24 h), the solution of the tested substance was removed from above the cells and 100 µl of neutral red solution in culture medium (50 µg/ml) was added to each well of the microplate. The cells were then incubated for 3 h, after which the dye was removed from above the cells. Then, using an elution mixture (50% ethanol, 49% distilled water, 1% glacial acetic acid), cell membranes were destroyed, releasing the dye accumulated in living cells. The dye concentration in the test and control samples was determined colorimetrically on the SYNERGY 2 reader at a wavelength of 540/450 nm, after shaking the plates for 3 minutes before measurement. Based on absorbance measurements, the percentage of viability of cells exposed to the xenobiotics tested was calculated in comparison with the control and the concentration of the test substance inhibiting the ability of cells to absorb the dye by 50% in comparison with the control (IC_{50}) was determined. IC_{50} values for each compound were calculated using the Gen5™ Data Analysis four-parameter logistic computer program from BIO-TEK INSTRUMENTS, INC.

Statistics

Statistical analysis of IC_{50} values was carried out using non-linear regression analysis at 95% confidence interval. A value of $p<0.05$ were considered to be statistically significant. The data were presented as the mean ± standard deviation (M±SD).

Results

Obtained values of IC_{50} (expressed both in mM and %) are collected in Table 2 and 3.

Analysing the cytotoxic effect of the tested compounds on respiratory system cells, it was found that in the vast majority of cases they have a stronger effect on the metabolic activity of cells assessed by the MTT test, while they exhibit a weaker cytotoxic effect in relation to the integrity of cell membranes, which was assessed by the NRU test. These differences are particularly visible when both types of cells are exposed to 2P, GA and EtOH (Figure 1).

Based on the conducted studies, it was also found that BEAS-2B cells were characterized by a significantly greater sensitivity to the effects of the tested compounds than A549 cells, which was particularly clearly observed when exposed to 1P, GA, DDAC, NaDCC and MAF in both tests (Figure 2).

Comparing the IC_{50} values determined for substances included in surface disinfection preparations, obtained on two cell lines derived from the respiratory system (A549 and BEAS-2B), it was found that DDAC and GA were the most toxic to A549 and BEAS-2B cells. Sodium diisocyanurate (NaDCC) and potassium monopersulfate (KPMS) were slightly less cytotoxic. The alcohols tested

Table 2. IC_{50} values [mM] of tested substances on A549 and BEAS-2B cells. Data are presented as the mean ± SD.

Substance	A549		BEAS-2B	
	MTT	NRU	MTT	NRU
1P	353.98±14.69	360.81±15.35	151.95±68.10	168.08±71.66
2P	325.18±53.99	565.26±31.14	258.77±37.02	364.79±63.67
GA	0.76±0.07	0.92±0.57	0.026±0.001	0.037±0.020
FenEtOH (in DMSO)	11.65±0.99	14.84±0.82	9.91±1.02	9.51±0.35
EtOH	299.55±95.19	463.02±4.58	243.98±23.17	363.21±13.11
DDAC	6.08E-06±1.01E-06	8.46E-06±1.52E-06	3.15E-06±1.25E-07	3.02E-06±4.10E-07
NaDCC	1.06E-03±8.89E-05	1.05E-03±2.03E-04	4.23E-04±1.83E-04	5.21E-04±7.13E-05
KPMS	6.90E-03±8.34E-04	7.51E-03±6.53E-04	6.86E-03±5.36E-04	9.67E-03±1.51E-03

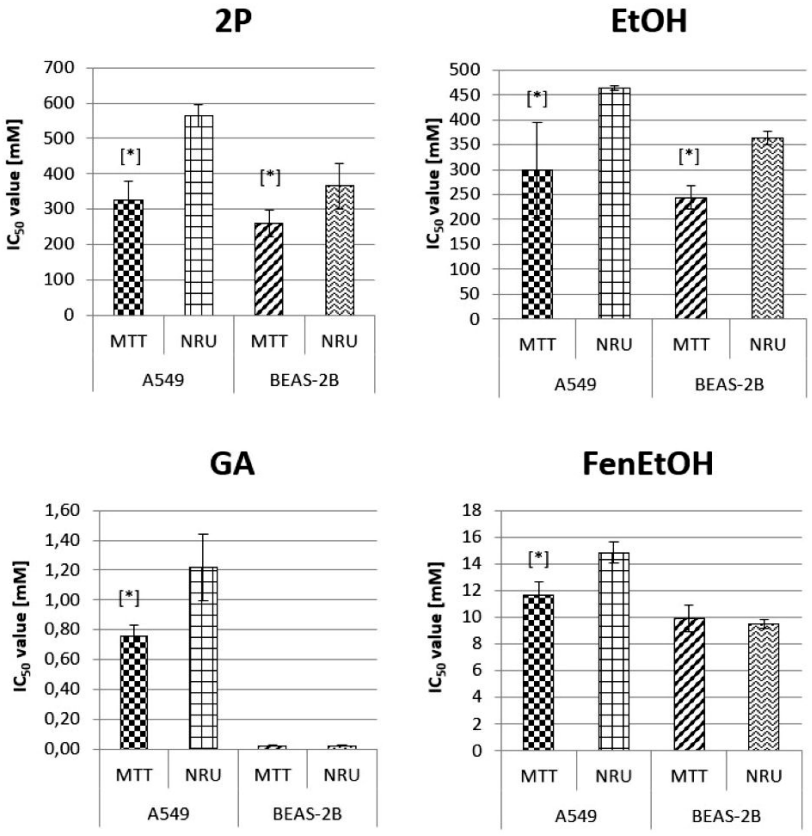

Figure 1. Comparison of IC_{50} values [mM] of 2P, EtOH, GA and FenEtOH experimentally determined on the basis of tests assessing cell metabolic activity (MTT) and cell membrane integrity (NRU) after exposure of A549 and BEAS-2B cells. Data are presented as the mean ±SD of three separate experiments. IC_{50} values of 2P, EtOH, GA and FenEtOH determined in MTT test on A549 cells were statistically significant ($p<0.05$) lower compared to NRU test. The same phenomenon was observed on BEAS-2B cells only for IC_{50} of 2P and EtOH.

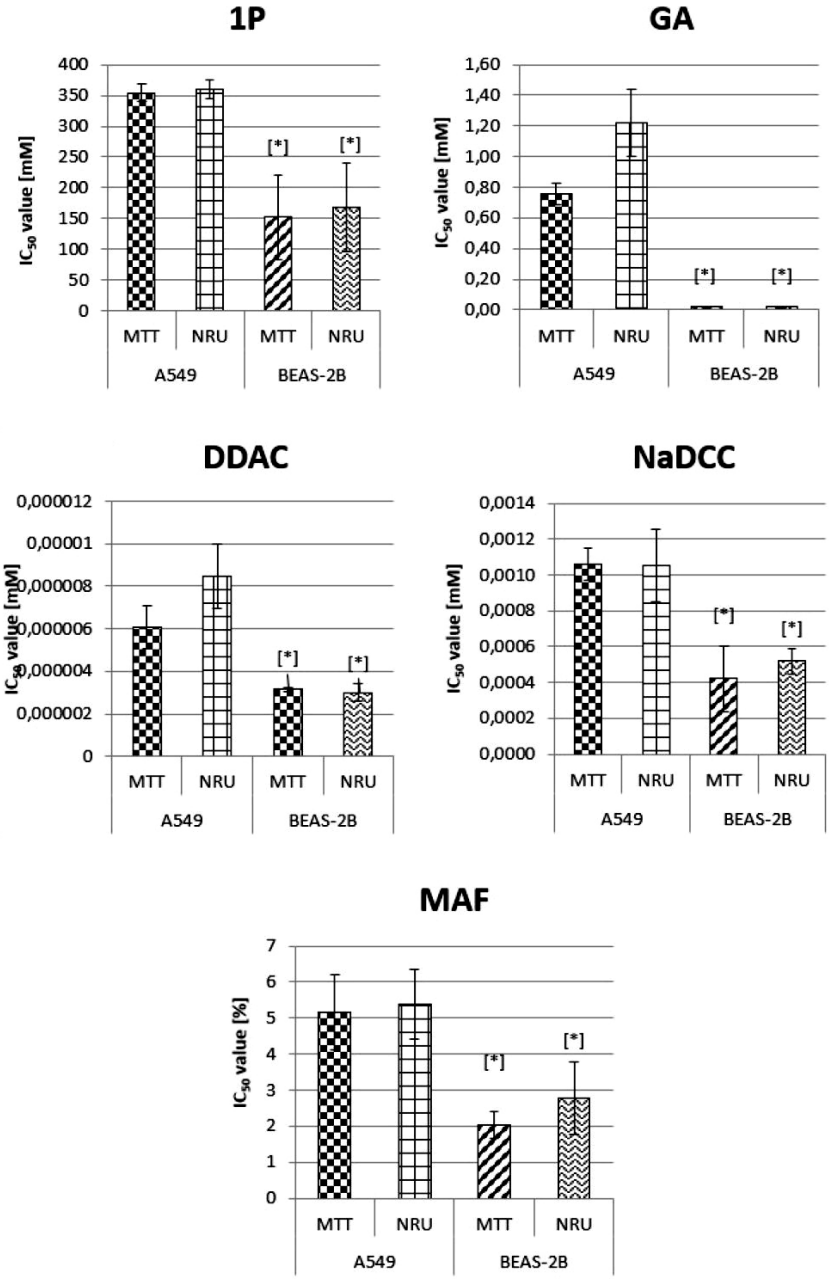

Figure 2. Comparison of IC_{50} values [mM] or [%] of 1P, GA, DDAC, NaDCC and MAF experimentally determined after exposure of A549 and BEAS-2B cells based on the assay assessing cell metabolic activity (MTT) and cell membrane integrity (NRU). Data are presented as the mean ±SD. IC_{50} values of 1P, GA, DDAC, NaDCC and MAF determined on BEAS-2B cells in both tests were statistically significant ($p<0.05$) lower compared to the same tests results performed on A549 cells.

Table 3. IC_{50} values [%] of tested substances/mixture on A549 and BEAS-2B cells. Data are presented as the mean ± SD.

Substance	A549		BEAS-2B	
	MTT	NRU	MTT	NRU
1P	2.087±0.365	1.419±0.656	1.141±0.402	0.871±0.548
2P	3.819±0.610	3.395±0.595	2.174±1.189	2.645±0.952
GA	6.862E-03±6.576E-04	8.349E-03±5.174E-03	2.339E-04±8.900E-06	3.304E-04±1.784E-04
FenEtOH (in DMSO)	0.1454±0.0124	0.1850±0.0114	0.124±0.013	0.119±0.004
EtOH	6.317±0.459	5.406±0.684	4.079±1.660	3.351±1.059
DDAC	4.401E-04±7.282E-05	6.126E-04±1.103E-04	2.279E-04±9.059E-06	2.185E-04±2.972E-05
NaDCC	0.0163±0.0014	0.0162±0.0031	0.0065±0.0028	0.0080±0.0011
KPMS	0.0580±0.0070	0.0632±0.0055	0.0577±0.0045	0.0813±0.0127
MAF	5.158±1.031	5.389±0.975	2.023±0.373	2.778±1.019

as part of the project, which are used as ingredients of disinfectants: 1P, 2P and EtOH, were characterized by lower toxicity to both A549 cells (Figure 3) and BEAS-2B cells (Figure 4). Comparing the IC_{50} values converted to percentage concentration obtained for the individual components of the ready-made, commercially available surface disinfectant MAF with the IC_{50} value determined for the mixture itself, its significantly lower toxicity to A549 cells was found in comparison with its tested active ingredients. MAF also had a weaker cytotoxic effect than both of its tested components on BEAS-2B cells, but the difference in cytotoxicity was not as high as in the case of exposure to A549 cells (Figure 5).

Discussion

The differences in the sensitivity of cells derived from different cell lines to the cytotoxic effects of disinfectant components found in this study are consistent with the observations made by Christen *et al.* (2017), who assessed the cytotoxic effects of disinfectants on zebrafish (Danio rerio) liver cells (ZFL) and human liver cells (Huh7 line) using the MTT test and found, among other things, greater sensitivity to glutaraldehyde in ZFL cells compared to Huh7. Based on their own studies, Sagripanti and Bonifacino (2000) classified glutaraldehyde to the group of highly toxic compounds, which confirms the results obtained in this study. They determined the GA concentrations causing a reduction in the number of cells by half on individual cell lines at the level of: 0.069±0.019 mM (L929); 0.059±0.012 mM (SC-1); 0.092±0.013 mM (VERO); 0.029±0.004 mM (H9) and 0.007±0.001 mM (CEM-SS), which is fully consistent with the results of our study, where a value of 0.0233±0.001 mM was obtained on BEAS-2B cells. Similarly, cytotoxicity studies were conducted by Iwasawa *et al.* (2011) for mixtures containing GA at a concentration of 2.25% and 3.5%, OPA at a

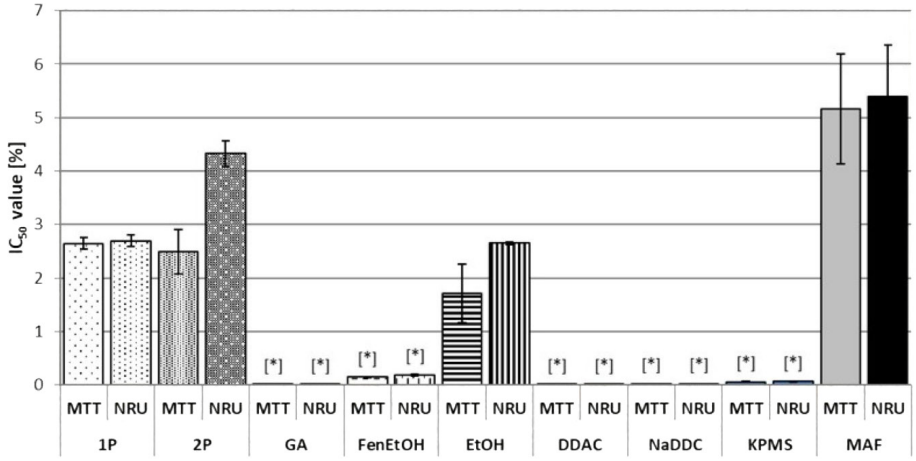

Figure 3. Comparison of the cytotoxic effect of the tested disinfectant components and the ready-made surface disinfection preparation on A549 cells based on the IC_{50} [%] values determined in the MTT and NRU tests. IC_{50} values of GA, FenEtOH, DDAC, NaDCC and KPMS determined on A549 cells in both tests were statistically significant ($p<0.05$) lower compared to the same tests results for 1P, 2P, EtOH and MAF.

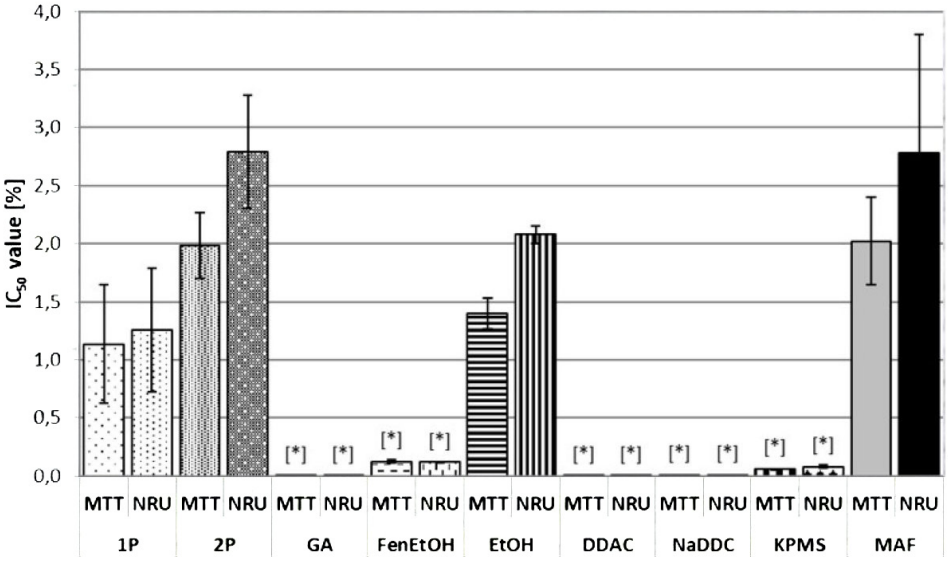

Figure 4. Comparison of the cytotoxic effect of the tested disinfectant components and the ready-made surface disinfection preparation on BEAS-2B cells based on the IC_{50} [%] values determined in the MTT and NRU tests. IC_{50} values of GA, FenEtOH, DDAC, NaDCC and KPMS determined on BEAS-2B cells in both tests were statistically significant ($p<0.05$) lower compared to the same tests results for 1P, 2P, EtOH and MAF.

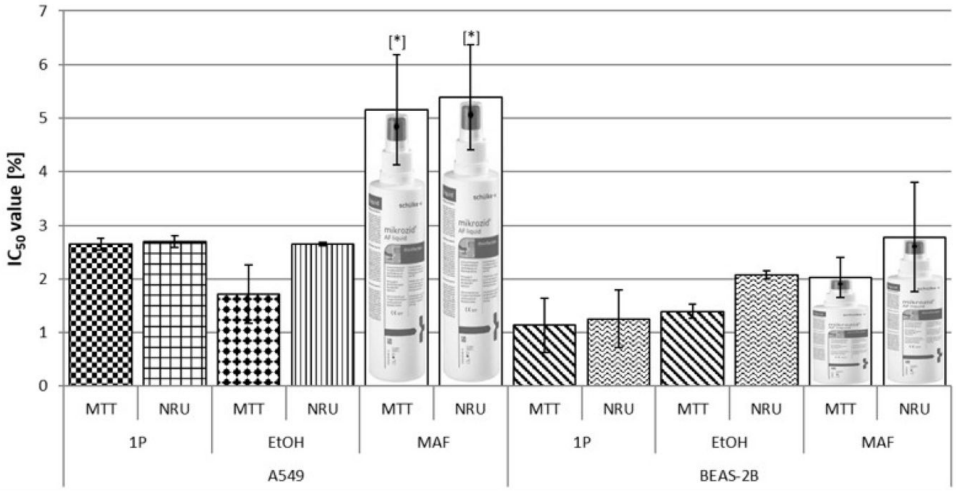

Figure 5. Comparison of the IC_{50} values [%] of Mikrozid AF (MAF) with the IC_{50} values [%] determined for its active ingredients: propan-1-ol (1P) and ethanol (EtOH) determined in A549 and BEAS-2B cells in the MTT and NRU tests. Data are presented as the mean ±SD. IC_{50} values of ready-for-use commercially available disinfectant mixture (MAF) determined on A549 cells were statistically significant ($p<0.05$) higher compared to the same tests results obtained for its active ingredients. This phenomenon was not observed on BEAS-2B cells.

concentration of 0.55% and for glutaraldehyde alone. The results showed that the OPA-containing product was less toxic than the GA-containing products and GA alone for the four cell lines tested (SIRC rabbit corneal epithelial cells; Chang human conjunctival cells; FRSK rabbit skin cell line; and HeLa S3 cells derived from human cervical cancer. They found that the minimum concentration that caused the majority (>75%) of all cells tested was 0.035% (100-fold dilution) of the 3.5% GA formulation; 0.0225% (100-fold dilution) of the 2.25% GA formulation; and concentrations ranging from 0.55% to 0.11% (neat to 5-fold dilution) of the 0.55% OPA formulation. The concentrations at which no cytotoxic effects were observed were: 0.0007% (5000-fold dilution) of GA3.5%; 0.00045% (5000-fold dilution) of GA2.25%; 0.0055% (100-fold dilution of OPA0.55% and 0.001% of glutaraldehyde alone at 48-hour exposure, which is consistent with the IC_{50} values for glutaraldehyde obtained in this work at 0.007% in the MTT test at 24-hour exposure on the A549 cell line.

IC_{50} values obtained in the MTT and NRU test for 2P in this study are consistent with the results of Nordin *et al.* (1991) obtained on the established L929 fibroblast cell line.

Differences observed between reactivity of used cell lines, indicating greater sensitivity of BEAS-2B cells to the cytotoxic effects of 1P, GA, DDAC, NaDCC and MAF, are probably related to the origin of the studied cell lines. A549 and BEAS-2B are both human lung epithelial cell lines, but A549 cells are derived from lung adenocarcinoma, while BEAS-2B is an adenovirus 12 SV40 transformed immortalized line from normal bronchial epithelium, possibly more representative of normal respiratory epithelium. The observed phenomenon is consistent with the results of the study by Zhang *et al.* (2017) on the evaluation of whole cigarette smoke induced oxidative stress in A549 and BEAS–2B cells. Difference between the two cell lines was also noted during comparison of the DNA damage response in BEAS-2B and A549 cells exposed to titanium dioxide nanoparticles (Biola-Clier *et al.*, 2017). On the other hand, the human primary bronchial epithelial cells (NHBE) responded differently to titanium dioxide nanoparticles than both the lung epithelial cell lines A549 and BEAS-2B (Ekstrand-Hammarström *et al.*, 2011). On the contrary, no differences between cell lines were found in the determination of cytotoxicity and genotoxicity of biogenic silver nanoparticles in A549 and BEAS-2B cells (Muhamad *et al.* 2022). Proper characterization of the responses of different respiratory cell types to chemicals is therefore essential to ensure that the selected cell line yields results relevant to *in vivo* conditions.

Mikrozid AF liquid is a formulation intended for disinfection of surfaces, equipment and medical supplies. The product was developed without the use of aldehydes. It is characterized by high effectiveness in a short time of action. It has wide material compatibility and can be used for disinfection of various materials and surfaces, for example furniture and equipment in medical offices. Mikrozid AF liquid is a quick-drying preparation, does not leave streaks, has a pleasant smell. The product was developed on the basis of alcohols. It is ready to use. It works perfectly in quick disinfection of medical devices in the area of increased risk of infection. A great advantage of the product is the short time of action, which allows for immediate disinfection of treatment sets including adjacent surfaces, couches used by patients, operating tables, work surfaces. Mikrozid AF liquid is also used for surfaces in the food section. The preparation has bactericidal, fungicidal, virucidal effects (including HBV, HCV, HIV, Adeno, Rota, Noro, Papova SV40, Polio, Vaccinia). Considering that MAF is a mixture of EtOH and 1P (100 g of Mikrozid AF liquid contains: 25 g of EtOH (94%) and 35 g of 1P, the remaining ingredients are amphoteric surfactants added as wetting agents), which when applied to A549 respiratory cells were characterized by higher cytotoxicity than the tested mixture, it seems reasonable to investigate/check possible interactions between the

components and confirm the alleged antagonism (?) in terms of cytotoxic activity or other (reduced) the mixture bioavailability.

Conclusions

- Didecyldimethylammonium chloride and glutaraldehyde were most toxic on respiratory cells, while alcohols: propan-1-ol, 2-propanol and ethanol were least
- The tested substances and mixture had a greater effect on the metabolic activity of cells assessed by the MTT test than on the integrity of cell membranes assessed by the NRU test
- BEAS-2B respiratory cells were characterized by significantly greater sensitivity to the tested compounds than A549 cells
- Commercially available disinfectant mixtures, such as MAF, appear to be safe for humans while effectively preventing the spread of pathogenic microorganisms. This is inextricably linked to medical procedures and healthcare services, and is also essential in beauty and hairdressing salons, agriculture, food production, and animal breeding.
- Comparative studies of the cytotoxicity of individual active ingredients of MAF and the mixture itself indicate the possibility of interactions (antagonism) between the ingredients in terms of cytotoxicity assessed by MTT and NRU tests although reduced bioavailability of chemicals in mixture cannot be also ruled out
- The obtained research results will serve as a starting point for assessing the combined effects of two-component mixtures of the tested substances and for examining the interactions between individual active ingredients in disinfectants, as further studies are still needed to confirm this phenomenon.
- Further studies involving various cell lines and chemical compounds are warranted to better understand the underlying mechanisms responsible for differences between experimental models.
- Understanding the impact of disinfectant ingredients on human cells has important implications for workplace safety regulations and guidance on selecting disinfectant formulations. While protecting workers in the workplace and the general population by eliminating harmful microorganisms, they must also be safe for those who use them.

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