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COMPARATIVE INVESTIGATION OF TOXICOLOGICAL CHARACTERISTIC AND SPECIFIC BIOLOGICAL ACTIVITY OF THE MEDIUM-CHAIN ALIPHATIC MONO- AND DIPEROXYCARBOXYLIC ACIDS

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ABSTRACT: The comparative toxicological characteristic *in-vitro* and antimicrobial activity to perform stability tests of several linear medium-chain aliphatic monoperoxyacids (perC_8–C_{12}) and aliphatic diperoxyacid (diperC9) were studied. Substances of peroxide acids: perC_8, perC_{10}, perC_{12}, diperoxyazelaic acid (diperC_9) are in 5.6; 22.8; 64 and 1.6 times less toxic than reference substance - peracetic acid (perC_2). New knowledge concerning a comparative antimicrobial activity of studied medium-chain aliphatic peracids and their thermal sensitivity was obtained. These fundamental stability studies resulted in the emergence of safe antimicrobic agents. Nonanebis(peroxoic acid) and Peroxyoctanoic acid were proposed as an agent in the chemical disinfection prosses for the first time. Medium-chain aliphatic peracids were demonstrated to be very robust. Sufficiently high bactericidal activity was achieved after relatively short exposition times (from 10 min to 2 h for spores of *B. anthracoid*), at near-ambient temperature (20 °C). In this work, it has been demonstrated how stability studies can underpin rational design of antimicrobic agents that in turn lead to a both safer and economically viable chemical disinfection and sterilization process.

INTRODUCTION: Peracids are characterized by high oxidation potential and therefore are very reactive oxidizing species and antimicrobic agents [1, 2]. The peroxy bond is weak and can be cleaved readily, which results in low stability of peracids. Peracids can decompose spontaneously and explosively under thermal and can undergo uncontrolled decomposition, which can be catalyzed by organic or metal compounds [3]. Therefore, peracids are treated as explosive materials and are covered by special regulations for shipping andstorage [3, 4].

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The shorter the alkyl chain, the more unstable the peracid is. This is the result of a higher percentage share of active oxygen in the molar mass of the peracid. The average dissociation energy of the peroxy bond of peracids is low, which causes the lowest stability in the group of organic peroxides. Aromatic Perbenzoic acid caused skin tumors in mice, but it is safer than peracetic or performic acid [4]. However, there are no appropriate results for safety measures for this species. Short-chain aliphatic peracids are miscible with water while the longer-chain (C_6 and higher) are not. As a result, short-chain peracids exhibited low toxicity on animals, and longer-chain is non-toxic and non-irritant. The most popular peracids are the short-chain, and the hazard of handling these hazardous materials limits their commercial application. For example, the transport and storage of peracetic acid is prohibited [5, 6].

High stability of the dodecanebis (peroxoic acid) at room temperature and non-shock sensitivity was confirmed with DSC [7]. The stability tests for medium-chain aliphatic monoperacids (C_6–C_{12}), which can be the safe alternative to very reactive short-chain analogs also was presented [8, 9].

Replacing short-chain peracids with less hazardous medium-chain peracids may lead to an economically viable process. Moreover, the application of a more effective antimicrobic analog, which minimizes the potential for chemical accidents, including explosions, is in compliance with the idea of green chemistry.

Among the variety of biologically active compounds, which are promising for development new antimicrobial pharmacotherapeutics, antiseptic and disinfection products, peroxycarboxylic acids, in particular, aliphatic acids especially attract the attention [10]. They are characterized by activity against a broad spectrum of microorganisms; they have a germicidal character of action, the slow formation of resistant strains, relative safety for the medical staff and patients, ecological safety, sufficient storage stability. Besides that, they have a solubility in water and lipids, compatible with many excipients and active medical products; they are tolerant to the construction materials and medical devices and have an optimal ratio of "efficiency-flowrate-the price". However, there are a few amounts of dates regarding the toxic properties of products of these classes, which significantly inhibit the identification of priority areas for large-scale targeted studies on the subject of their introduction into medical practice.

The objective of this study has been the investi-gation of a comparative toxicological characteristic of four medium-chain aliphatic peroxyacids: peroxyoctanoic ($perC_8$), peroxycapric ($perC_{10}$), peroxylauric ($perC_{12}$), diperoxyazelaic acids ($diperC_9$) and peracetic acid ($perC_2$) as a reference substance.

MATERIALS AND METHODS: Safety Note: Caution! Peroxyacetic acid ($perC_2$) solutions cause severe irritant to eyes, skin, and mucous membranes [3, 4]. Both ($perC_2$) and H_2O_2 are strong oxidizing agents that are completely incompatible with easily oxidized substances and can form explosive mixtures with them; therefore, their concentrated solutions should be handled very carefully and should not be mixed with either reducing agents or organic substances, including solvents.

Peroxyacetic acid ($perC_2$) were purchased from Sigma-Aldrich (CAS Number 79-21-0). Peracetic acid solution purum, ~39% in acetic acid (RT). As impurities were ≤6% H_2O_2, ~45% acetic acid; density 1.15 g mL^{-1} at 20 °C.

All solutions were prepared using double distilled water prepared in a quartz distiller, and all chemicals were of analytical grade.

The $perC_2$ solutions of appropriate concentrations were prepared from their stock solutions (38.4% for $perC_2$ and 5% H_2O_2). The concentration of $perC_2$ stock solution was analyzed to be 5.0 M using the conventional method originally proposed by Greenspan and Mackellar [11]. The concentration of H_2O_2 coexisting in the $perC_2$ stock solution was determined to be 1.5 M by the same method.

Procedure: The sample of peracid is accurately weighed and placed in a 500 mL. Erlenmeyer flask containing 150 mL of 5% sulfuric acid and sufficient cracked ice to maintain a temperature of 0 °C to ± 10 °C. An adequate sample is chosen, when possible, to give approximately 40 mL of thiosulfate titration. Three drops of ferroin indicator are added, and the flask contents are titrated with *c*(Ce(IV)) 0.1 mol L^{-1} (0.1 N) ceric (IV) sulfate to the disappearance of the salmon color of the indicator. Then 10 ml of the 10% potassium iodide solution is added, and the liberated iodine is titrated with 0.1mol L^{-1} sodium thiosulfate. A starch indicator is added near the endpoint for the thiosulfate titration.

Calculations:

% H_2O_2 = (*V*, mL Ceric sulfate × *c*(Ce(IV)) × 17) / (10 × sample weigth);

% Peracid = (*V*, mL $Na_2S_2O_3$ × *c*($Na_2S_2O_3$) × equivalent mass of per acid) / (10 × sample weigth)

Octanoic, Decanoic, Dodecanoic, and Azelaic acid (Nonanebis (oxoic acid)) acids were purchased from Sigma-Aldrich. Glacial acetic, $MgSO_4 \times 7H_2O$, and H_2SO_4 95 wt% were of analytical grade; 35 wt% H_2O_2 solutions in water (Perhydrol) and

50 wt% hydrogen peroxide of medical quality were purchased from "Inter-Synthesis" LLC, Borislav (Ukraine).

65wt% solution of hydrogen peroxide was prepared from a concentrated solution of hydrogen peroxide. A concentration of hydrogen peroxide solution was obtained by means of evaporation of water under reduced pressure on a water bath (45 to 50 °C).

Procedure for the Preparation of Concentrated Hydrogen Peroxide: A 500 mL distilling flask is provided with a standard male ground joint, onto which is placed a female glass cap, equipped with a distillation capillary. The side tube of the capillary is connected with ground joints to a spiral condenser, which empties into a receiver of about 200 mL capacity. After the introduction of 180 mL of Perhydrol (stored in bottles coated with paraffin wax), the flask is placed on a water bath (45 to 50 °C), and the material is distilled over a period of about 3.5 hours at a pressure of 16 to 22 mm. Thus, about 150-160 mL of water and some hydrogen peroxide are removed. The residue contains approximately 96% H_2O_2. A 0.1 mol L^{-1} solution of sodium thiosulfate was prepared from the standard titer fixanal.

Melting points of peracids were determined using the EZ-Melt apparatus (maximum temperature range: 20-400 °C). Measurements were performed in a capillary using 5 mg of each sample. The temperature range was set as 28-65 °C in a heating rate of 0.5 °C min^{-1}.

Synthetic Procedure: Diperoxyazelaic acid was synthesized by a known Swern's method [12] according to the scheme (in the present sulfuric acid):

$$HO(O)C(CH_2)_7C(O)OH+H_2O_2 = HOO(O)C(CH_2)_7C(O)OOH + H_2O$$

Procedure for the Preparation of Diperoxyazelaic Acid (Nonanediperoxoic Acid): In 250 mL round-bottomed flask equipped with mercury sealed stirrer, 10 g of azelaic acid (0.0531 mol) was dissolved in 95 % sulfuric acid (25 g, 0.255 mol) with good stirring. The reaction mass was cooled to 15 °C using an ice-water bath. To this, 65% hydrogen peroxide (11 g, 0.323 mol) was added dropwise while maintaining the internal temperature at 15 to 20 °C. After the addition, the reaction mass was further stirred for 5 h at 15 to 20 °C. Then, 50 mL half saturated aqueous ammonium sulfate solution (35 g /100 g water) was added to the reaction mass at 0 °C.

The white solid was filtered and washed with cold half saturated ammonium sulfate solution (4 × 10 mL). The crude product obtained was dried under vacuum at room temperature to give the final product (9.2 g, 78% yield).

Diperoxyazelaic acid (nonanebis (peroxoic acid)), $T_{mp.}$ = 90-90, 5ºC (with decomp.), the content of active oxygen species (AOC) 14.2%; pKa_1 = 8.08, pKa_2 = 9.19.

The purity of peroxy acids, as well as the content of peroxy groups in the post-reaction mixtures, was determined by iodometric titration. A weighted sample (*ca.* 0.100–0.200 g) and 20 mL of glacial acetic acid were introduced to the 250 mL Erlenmeyer flask. The flask was purged with argon for 25 seconds, and a pinch of sodium iodide was added. After closing, the flask was placed in the dark for 30 min. Then 20 mL of distilled water was added, and the mixture was titrated by the 0.1 M aqueous solution of sodium thiosulphate. A molar content of peroxy groups was calculated according to an equation

$$n = 0.5cV_T \text{ [mol]}$$

where: 0.5 – coefficient calculated based on the stoichiometry of the reactions $2I^- + 3H^+ + OOH^- \rightarrow I_2 + 2H_2O$ and $I_2 + 2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2I^-$, c – molar concentration of the titrant [mol L^{-1}], V_T – the volume of the titrant [mL].

Calculation: AOC = (Burette reading) (Normality of $Na_2S_2O_3$) (0.008) (100)% / (Sample weight).

To confirm the stability of Nonanediperoxoic acid, one sample was kept in a self-sealing bag at room temperature (30–35 °C). The active oxygen content of the sample was determined by iodometric titration after every 10-15 days. It was found that it retains its active oxygen content over a period of 50 days (14.2-14.1%). There was no change observed in physical appearance, too. This confirms the stability of DPAA at room temperature.

General Procedure for the Synthesis of Monoperacids: Peroxyoctanoic, peroxydecanoic, and peroxydodecanoic acids were synthesized based on the procedure described in the literature [13].

Carboxylic acid (20.0 g, 0.10–0.17 mol) dissolved in 95 wt% sulphuric acids (40.0 g) was introduced into a high 250 mL beaker, equipped with a mechanic stirrer, and placed in an ice bath to cool (10 °C). After stabilization of the temperature, the 50 wt% aqueous solution of hydrogen peroxide (1.5 eq.) was carefully dropwise for 10 min, taking care not to exceed the temperature of the mixture above 30 °C. After the addition of the oxidant, the mixture was stirred at 1000 rpm for 50 min. Next, ice water (*ca.* 150 mL) was slowly added to the beaker, and peracid crystallized as a white solid. The content of the beaker was transferred to a separating funnel and extracted with diethyl ether (5 × 30 mL). The organic phase was washed with water (20 mL) and dried over anhydrous $MgSO_4$. After filtration, the filtrate was carefully evaporated on a vacuum rotary evaporator at 25 °C. Crude peracid was recrystallized from petroleum ether (10 mL/1 g of the peracid) at −20 °C. The product was then filtered off under vacuum and washed with cold petroleum ether (10 mL). The yields of peracids were in the range of 90–95%. Characteristics obtained peracids are shown in **Table 1**.

TABLE 1: CHARACTERISTICS OBTAINED PERACIDS

Peracid	Melting point (dec.) (°C)	Purity[a] (wt%)
$C_7H_{15}COOOH$ ($perC_8$)	+ 31.5–32.6	98
$C_9H_{19}COOOH$ ($perC_{10}$)	+ 42.7–43.3	99
$C_{11}H_{23}COOOH$ ($perC_{12}$)	+ 49.5–50.5	99
$HO_3C(CH_2)_7CO_3H$ ($diperC_9$)	+ 90.0–90.5	98

[a] Calculated based on the iodometric titration

In order to select the least toxic compounds from the line of technologically equivalent drugs on the stage of "test-tube" laboratory synthesis and also for determination indicative values parameters of acute toxicity prediction, forecast of toxic doses and concentration before carrying out the deployed standard of toxicological studies on animals mitochondrial test system is used [14].

The antimicrobial (bactericidal) activity of the synthesized peroxyacids was studied by the suspension method and the method of cambric test objects [15, 16]. The antimicrobial activity testing of peroxyacids by the method of cambric test objects was carried out on standard test strains of the following types: *St. aureus* 906, *E. coli* 1257, *Ps. aeruginosa* ATCC 27853, *Bacillus subtilis* 7, and *Bacillus anthracoides* 1312.

In the determination of the peracids antimicrobial activity by recommendations of the society hygienists and microbiologists in Germany used WHO reference strains and museum cultures: *E. coli* (ATCC 25922), *Ps. aeruginosa* (ATCC 27853), *St. aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633) as test culture. In the experiments microorganism's suspension was used, which ultimate standard density was 1×10^8 CFU/mL (in quantitative suspension method by recommendations of the society hygienists and microbiologists in Germany) and 2×10^9 CFU/mL (in studies using the method of cambric test objects).

RESULTS AND DISCUSSION: From dates which are shown in **Table 2**, peroxylauric acid ($perC_{12}$) has the lowest toxicity, and the highest – peroxyacetic acid (the substance of comparison, $perC_2$).

TABLE 2: COMPARATIVE TOXICOLOGICAL ACTIVITY OF MEDIUM-CHAIN ALIPHATIC PEROXYACIDS [17]

S. no.	Substances of peroxycarboxylic acids	No. of carbon atoms in the molecule, C_n	LD_{50}
1	Peroxyacetic ($perC_2$) (reference substance)	2	25±3
2	Peroxycaprylic acid ($perC_8$)	8	140±1
3	Peroxycapric ($perC_{10}$)	10	570±1
4	Peroxylauric ($perC_{12}$)	12	1600 ±1
5	Diperoxyazelaic ($diperC_9$)	9	40±1

Note: LD_{50} – dose of peroxide acid substances in mcg, which has a 50% inhibitory effect on mitochondria.

Substances of Peroxide Acids: $perC_8$, $perC_{10}$, $perC_{12}$, diperoxyazelaic acid ($diperC_9$) are in 5.6; 22.8; 64 and 1.6 times less toxic than the substance of comparison – peracetic acid [17]. Since per-oxyacids $perC_8$, $perC_{10}$, $perC_{12}$ exhibit the lowest toxic effect among investigated acids, they are inducing particular interest for further detailed pharmacological and toxicological studies.

Specific Biological Activity of Medium-Chain Carboxylic Acids:

Antimicrobial Activity of Medium-Chain Aliphatic Mono- and Diperoxycarboxylic Acids: Results of comparative investigation of antimicrobial activity planned series of medium-chain aliphatic peroxycarboxylic acids are shown in **Table 3**.

Lengthening of an alkyl radical in the series of tested monoperoxyacids leads to a dramatic decrease in their bactericidal effects, which is most likely due to the condition of low active micelles' development in experimented condition. This, in particular, was demonstrated by the fact, that bactericide action of peroxycapric acid is reached at a concentration 0.01%, which corresponds numerically to the value of CCM which earlier has been found for it (critical concentration of micelle formation) [19, 20]. With further increase of solution C_{10} critical decrease of efficacy is observed, which is explained by including more and more molecules in small reactive micelles and consequently less their parts are in free condition, and they don't provide microbicidal effect at the proper level **Table 4.** In all likelihood, by this can be explained the strong neutralizing action on bactericidal activity of peroxidecapric acids of proteins charge, when, probably, in experiment condition, the developing mixed micelles "protein – peroxyacid" take place.

TABLE 3: TEST RESULTS ON THE BACTERICIDAL ACTIVITY OF MEDIUM-CHAIN ALIPHATIC MONO- AND DIPEROXYCARBOXYLIC ACIDS (HAVE BEEN MADE ON THE RECOMMENDATIONS BY THE SOCIETY HYGIENISTS AND MICROBIOLOGISTS IN GERMANY) [18]

S. no.	Compound	Germs	w (wt%)					
			0.05	0.025	0.012	0.006	0.003	0.0015
1	perC$_2$, CH_3CO_3H	*S. aureus*	-	-	-	-	-	+
		E. coli	-	-	-	+	+	+
		P. aerug.	-	-	-	-	+	+
		B. subtilis	-	+	+	+	+	+
2	perC$_8$, $CH_3(CH_2)_8CO_3H$	*S. aureus*	-	-	+	+	+	+
		E. coli	-	+	+	+	+	+
		P. aerug.	-	-	+	+	+	+
		B. subtilis	-	+	+	+	+	+
3	diperC$_9$, $CH_3(CH_2)_7CO_3H$	*S. aureus*	-	-	-	-	+	+
		E. coli	-	-	-	+	+	+
		P. aerug.	-	-	-	+	+	+
		B. subtilis	-	+	+	+	+	+

Note: Microbial burden - 10^8 CFU /mL; «-» - microbial growth was not observed; «+» - the presence of microbial growth. Exposition of 5 min

TABLE 4: TEST RESULTS ON THE BACTERICIDAL ACTIVITY OF MEDIUM-CHAIN ALIPHATIC MONO- AND DIPEROXYCARBOXYLIC ACIDS (MICROBIAL BURDEN 2×10^9 CEL/ML)

S. no.	Compounds	Content,	*E. coli*					*S. aureus*				
		% (wt)	5'	10'	15'	30'	60'	5'	10'	15'	30'	60'
1	perC$_8$, $CH_3(CH_2)_6CO_3H$	0.001	+	+	-	-	-	+	-	-	-	-
		0.003	+	±	-	-	-	-	-	-	-	-
		0.005	+	-	-	-	-	-	-	-	-	-
		0.01	-	-	-	-	-	-	-	-	-	-
		0.01*	±	-	-	-	-	±	±	-	-	-
2	perC$_{10}$, $CH_3(CH_2)_8CO_3H$	0.01	+	-	-	-	-	+	+	-	-	-
		0.01*	+	+	+	+	+	+	+	+	+	+
		0.1	+	-	-	-	-	+	+	±	-	-
3	perC$_{12}$, $CH_3(CH_2)_{10}CO_3H$	0.03	+	+	+	-	-	+	+	-	-	-
		0.03*	+	+	+	+	+	+	+	+	+	+
4	diperC$_9$, $HO_3C(CH_2)_7CO_3H$	0.003	+	-	-	-	-	+	-	-	-	-
		0.005	+	±	-	-	-	-	-	-	-	-
		0.01	+	-	-	-	-	-	-	-	-	-
		0.01*	+	-	-	-	-	+	-	-	-	-

Note: «-» - microbial growth was not observed; «+» - the presence of microbial growth; *– proteins derivatives are 20%

Table 5 shows the results of investigation of sporicidal activity of peroxycarboxylic acids regarding anthracoid's spores both at the presence and without protein impurities. Results of sporicidal activity of monocarboxylic acids which have been obtained to give a reason to be ranged to the class of perspective sporicidal of the next product: diperC_9, which show deadly action on anthracoid spores in 0.2% concentration at 120 min exposure and 20% of protein impurities. The high activity is also inherent, and perC_8, which also have deadly action on anthracoid spores but at 180 min exposure.

TABLE 5: SPORICIDAL ACTIVITY OF PEROXYCARBOXYLIC ACIDS

Code	Protein Charge	w, wt-%	Vital activity of *Anthracoid's* spores after influence of them tested products					
			15'	30'	60'	90'	120'	180'
perC_8	w/p	0.1	+	+	+	+	+	-
	20% p	0.2	+	+	+	+	±	-
diperC_9	w/p	0.1	+	+	+	+	±	-
	20% p	0.2	+	+	+	+	-	-

Note: «+» - test-organisms vital; «-» - test-organisms unvital; w/p the absence of protein charge; 20% p – 20% protein charge

Results of comparative investigation bactericidal and sporicidal activities of peroxycarboxylic acids with Peracetic acid are shown in **Tables 6** and **7**. Comparative results of the investigation on bactericidal activity of peroxycarboxylic acids indicate their superior activity in comparison with perC_2 at different temperatures **Table 6**.

TABLE 6: COMPARATIVE BACTERICIDAL ACTIVITY OF PERACETIC ACID (PERC_2) AND SOME OTHERS PEROXYCARBOXYLIC ACIDS AT THE DIFFERENT TEMPERATURE

Code	*t*,°C	*w*, wt-%	*E. coli*						*S. aureus*				
			3'	5'	10'	15'	30'	60'	3'	5'	10'	15'	30'
perC_2	4	0.001	+	+	+	+	-	-	+	+	+	+	-
	20	0.001	+	+	+	+	-	-	+	+	+	+	-
	50	0.001	+	-	-	-	-	-	+	-	-	-	-
diperC_9	20	0.001	+	+	+	-	-	-	+	+	+	-	-
	50	0.001	+	+	±	-	-	-	+	+	±	+	-
perC_8	4	0.001	+	+	+	+	-	-	+	+	+	+	-
	50	0.001	+	+	+	+	-	-	+	+	+	+	-

Note: «+» - test-organisms vital; «-» - test-organisms unvital

As shown from **Table 6** at 20°C, the product of diperC_9 has 15 min advantages against a reference substance (perC_2), regarding both types of micro-organisms, and a product of perC_8 has no inferior action to Peracetic acid on bactericidal activity.

We note that sporicidal activity of perC_2 at the room temperature storage condition is dramatically decreased in the first month, whereas products of peroxycarboxylic acids save stable activity for 1 year. The results are shown in **Table 7**.

TABLE 7: COMPARATIVE SPORICIDAL ACTIVITY OF PEROXYACETIC ACID (PERC_2), PEROXYOCTANOIC (PERC_8) AND DIPEROXYAZELAIC ACID (DIPERC_9) AFTER 30 DAYS OF STORAGE

S. no.	Compound	*w*, wt-%	Exposure of products (min) to *B. anthracoides* spores	
1	perC_2	0.5	30'	180'
		1.0	5'	180'
2	perC_8	0.2	120'	120'
3	diperC_9	0.2	120'	120'

Thus, it can be concluded that the obtained results generally confirm earlier stated admission regarding the highest antimicrobial activity of peroxycarboxylic acids with a quantity of hydro-carbonic atoms in the aliphatic chain more than 7.

This conclusion is well conformed to the theoretical conception about physic-chemical reactivity capabilities as the root causes of the detection selectivity of their biological action in biological systems [20].

The acids have inherent biocidal activity. The most expressed bactericidal activity is featured for the next products: perC_8 and diperC_9. Compounds of perC_8 and diperC_9 stably retain their bactericidal

activity and physic-chemical properties for 1 month when stored in a cool place or under-cooling.

Protein impurities affect the bactericidal activity of products, pH, and the temperature of the working solution. In an acid medium (pH 6.0) and when the temperature rises (+50°C), the biocidal activity of peroxyacids is increased. The protein impurities decrease the bactericidal activity of products. Product of diperC_9 in a 0.2% concentration at room temperature makes a deadly action on anthracoid spores during 2 h exposure. The product of perC_8 at the same conditions destroys anthracoid spores during a 3-hour exposure.

Theoretically, dependence has been interpreted as "structure – physic-chemical properties – specific biological activity" in the rage of aliphatic medium-chain mono- and diperoxycarboxylic acids. In general, bactericidal activity is correlated with changing of real peroxyacids' redox-potentials (E_h), effective rate of constant in the model reaction of oxidation of the substrate, and the degree of ionization in the water solution of peroxide acids [19, 20].

Considering the satisfactory physic-chemical and also high bactericidal properties investigated per-oxycarboxylic acids for the further investigation of disinfection properties should be referred to the next products: diperC_9 and perC_8.

CONCLUSION: Increasingly stringent environ-mental regulations of technological processes, mostly with regard to safety, forced the modification of a number of preventive processes, in particular concerning chemical disinfection and sterilization processes. The results discussed above expanded and developed safe agents based on medium-chain peracids. The key development was to study comparative toxicological characteristics in vitro and to perform stability tests of several linear medium-chain aliphatic monoperoxyacids (perC_8–C_{12}) and medium-chain aliphatic diperoxyacid (diperC_9). Substances of peroxide acids: perC_8, perC_{10}, perC_{12}, diperoxyazelaic acid (diperC_9) are in 5.6; 22.8; 64 and 1.6 times less toxic than reference substance – peracetic acid (perC_2). This work delivered new knowledge concerning a comparative antimicrobial (bactericidal) activity of studied medium-chain aliphatic peracids and their thermal sensitivity.

These fundamental stability studies resulted in the emergence of safe antimicrobic agents. Nonanebis (peroxoic acid) and Peroxyoctanoic acid were proposed as an agent in the chemical disinfection processes for the first time. Medium-chain aliphatic peracids were demonstrated to be very robust. Sufficiently high bactericidal activity was achieved after relatively short exposition times (from 10 min to 2 h for spores of *B. anthracoid*), at near-ambient temperature (20 °C).

In summary, in this work, it has been demonstrated how stability studies can underpin the rational design of antimicrobic agents that in turn lead to a both safer and economically viable chemical disinfection and sterilization process.

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REFERENCES:

1. Awad MI, Denggerile A and Ohsaka T: Electroreduction of peroxyacetic acid at gold electrode in aqueous media. J Electrochem Soc 2004; 151: 358-63.
2. Luukkonen T and Pehkonen SO: Peracids in water treatment: A critical review. Critical Reviews in Environmental Science and Technology 2017; 47(1): 1-39.
3. Patnaik AA: Comprehensive Guide to the Hazardous Properties of Chemical Substances. John Wiley & Sons, Inc., 2007: 127.
4. Sanchez J and Myers TN: Peroxides and Peroxide Compounds, Organic Peroxides. Kirk-Othmer Encyclopedia of Chemical Technology 2000.
5. Klenk H, Götz PH, Siegmeier R and Mayr W: Organic peroxy compounds. Ullmann's encyclopedia of industrial chemistry. New York, John Wiley & Sons 2005.
6. Klenk H, Götz PH, Siegmeier R and Mayr W: Peroxy Compounds. Organic. Ullmann's Encyclopedia of Industrial Chemistry 2000: 332-36.
7. Patil VV and Shankarling GS: Nonanebis(peroxoic acid): a stable peracid for oxidative bromination of amino-anthracene-9,10-dione. Beilstein J Org Che 2014; 10: 921-28.
8. Patil VV, Gayakwad EM and Shankarling GS: Highly efficient and stable peracid for rapid and selective oxidation of aliphatic amines to oximes. New Journal of Chemistry 2015; 39(8); 6677-82.

9. Sitko M, Szelwicka A, Wojewodka A, Skwarek A, Tadasiewicz D, Schimmelpfennig L, Dziuba K, Morawiec-Witczakc M and Chrobok A: Perdecanoic acid as a safe & stable mediumchain peracid for Baeyer–Villiger oxidation of cyclic ketones to lactones. RSC Adv 2019; 9: 30012-23
10. Krasilnikov AP: Handbook on antiseptic. Moscow, High school, 1995: 172-75.
11. Greenspan FP and MacKellar DG: Analysis of Aliphatic Per Acids. Analytical Chemistry 1948; 20(11): 1061–1063.
12. Parker, WE, Witnauer LP and Swern D: Peroxides. IV.2 Aliphatic Diperacids. JACS 1957; 79(8): 1929-31.
13. Parker WE, Ricciuti C, Ogg CL and Swern D: Peroxides. II. Preparation, characterization and polarographic behavior of longchain aliphatic peracids. J Am Chem Soc 1955; 77(15): 4037-41.
14. Rotenberg YS: Study guide of biological activity by polarographic method. Moscow, Science 1973: 213-29.
15. GOST R: Chemical disinfectants and antiseptics. Disinfectants. Criteria and indicators for evaluating effectiveness. Moscow, Standartinform 2016.
16. Metody laboratornykh issledovaniy i ispytaniy dezinfektsionnykh sredstv dlya otsenki ikh effektivnosti i bezopasnosti. Moskva, Federalnyy tsentr gigiyeny i epidemiologii Rospotrebnadzora 2010.
17. Blazheyevskiy MY, Chernykh VP and Kovalenko SM: Comparative toxicological characteristic of high aliphatic per acids. Scientific basis of the development new drugs, Papers of scientific session of the department of Chemistry NAS of Ukraine. Kyiv, Base 1998: 482-85.
18. Krasilnikov AP: Handbook on antiseptic. Moscow, High School, 1995: 172-75.
19. Albert A: Selective toxicity. Physics and chemistry of theory. Moscow, Medicine, 1989: part.2.
20. Blazheyevskiy MY and Chernykh VP: Antimicrobial activity of aliphatic peroxycarboxylic acids compared to their oxidizing capacity and the degree of ionization in water solution. Physiol Active Substances 1999; 2(28): 58-65.

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