DRUG BIOCHEMISTRY

EVALUATION OF MUTAGENIC PROPERTIES OF $2(C_6H_{15}N_2O_2)+2I_3-H_2O$ IODINE COORDINATION COMPLEX IN BACTERIAL REVERSE MUTATION TEST

ARDAK BISENBAYEVNA JUMAGAZIYEVA^{1*}, ZHANAR ALIBAYEVNA ISKAKBAYEVA¹,
AUYES NAURYZBEKOVNA MYRZABAYEVA¹, NATALYA ALEXANDROVNA SULDINA¹,
NATALIYA ALEKSEEVNA PARETSKAYA¹, UBAIDILLA MAKHAMBETOVICH DATKHAYEV²,
ELENA VLADIMIROVNA FLISYUK³ and ALEKSANDR IVANOVICH ILIN¹

¹Laboratory of Microbiology, JSC Scientific Center for Anti-Infectious Drugs,
75B al-Farabi Ave., A15E3G8 Almaty, Kazakhstan

²Department Pharmaceutics, JSC Asfendiyarov Kazakh National Medical University,
94 Tole bi St., A05H2A6 Almaty, Kazakhstan

³Department of Dosage form Technology,
Saint-Petersburg State Chemical Pharmaceutical Academy,
14 Popov St., Saint-Petersburg, Russia

Abstract: $2(C_6H_{15}N_2O_2)+2I_3-H_2O$ iodine coordination complex is a new pharmaceutical substance that has high antibacterial activity against both sensitive and multidrug-resistant strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. Mutagenicity of $2(C_6H_{15}N_2O_2)+2I_3-H_2O$ iodine coordination complex was assessed using *Salmonella/microsome* (Ames) test. Tester strains used were *Salmonella typhimurium* TA98, TA100, TA1535, TA 1537, and *Escherichia coli* WP2uvrA. The mutagenic activity was determined both in the absence or presence of S9 mixture. The study has shown that test item did not cause any increase in the number of his⁺ revertants in *S. typhimurium* and trp⁺ revertants in *E. coli* WP2uvrA strains in the presence or absence of S9-mix, compared to the controls. Under the conditions of this test and according to the criteria set for the evaluation of the test results, the $2(C_6H_{15}N_2O_2)+2I_3-H_2O$ iodine coordination complex did not show mutagenic activity in the *S. typhimurium* and *E. coli* plate incorporation assay.

Keywords: iodine coordination complex, Ames test, mutagenicity

Coordination compounds of iodine including alkali metals, halides and various biologically active molecules (amino acids, peptides and carbohydrates) are of great interest in terms of the variety of their pharmaceutical properties. For example, Zubenko et al. had written about high antiviral activity of coordination compound based on molecular iodine against the influenza A virus (1).

Another coordination complex FS-1 relates to iodine with bioorganic ligands, magnesium, and lithium cations possess broad antimicrobial activity against antibiotic-resistant and antibiotic-susceptible Gram-positive and Gram-negative bacteria, mycobacteria, fungi, and viruses (2-8).

$2(C_6H_{15}N_2O_2)+2I_3-H_2O$ is the original iodine-containing complex, its structure was registered in

The Cambridge Crystallographic Data Centre (CCDC, [q1036668](#)). Recently, it was shown that $2(C_6H_{15}N_2O_2)+2I_3-H_2O$ iodine coordination complex has various biological activities. For instance, the coordination complex is effective against *S. aureus*, *E. coli*, *P. aeruginosa*, etc. bacterial strains with multidrug resistance (9). In addition, $2(C_6H_{15}N_2O_2)+2I_3-H_2O$ iodine coordination complex shows synergy effect with several antibiotics that evaluated by decrease MIC concentration of both, iodine coordination complex and antibiotic used. However, being a new original substance, safety assessment is one of the necessary pre-clinical tests.

The number of countries in which multiple-resistance to antibiotics is causing failures in the treatment of infectious diseases is increasing annu-

* Corresponding author: e-mail: r_dawa@mail.ru

ally (10). Therefore, many researchers are focusing on identifying novel antibacterial agents. The development of a new drug by chemical synthesis should be continued as they are of primary importance to maintain the effectiveness of antimicrobial treatment. However, as any substance received by chemical synthesis, their safety must be scientifically assessed, thus it may be valuable to assess their safety for continuous use (11). The bacterial reverse

mutation test developed by Ames et al. is a biological assay to assess the mutagenic potential of chemicals. It serves as a quick assay to estimate the mutagenic, premutagenic and carcinogenic potential of a compound, and a positive test indicates that the chemical might act as a mutagen or carcinogen (12). Herein, we tried to estimate the mutagenicity of $2(C_6H_{15}N_2O_2) \cdot 2I_3 \cdot H_2O$ iodine coordination complex by Ames assay.

Table 1. $2C_6H_{15}N_2O_2 \cdot 2I_3 \cdot H_2O$ iodine coordination complex and its properties.

Test item name:		$2C_6H_{15}N_2O_2 \cdot 2I_3 \cdot H_2O$ iodine coordination complex	
CAS No.:		39392-86-4	
Summary formula:		$2(C_6H_{15}N_2O_2) \cdot 2(I_3) \cdot H_2O$	
Structure:
Composition:	Component type:	CAS No.:	Concentration (%):
	Main components:	7553-56-2 Iodine 17023-24-4 Potassium iodide 657-27-2 Lysine hydrochloride	43.34 31.92
Molecular weight:			1073.81
Physical state at 20°C and 101.3 kPa:			Solid
Form:			Powder
The way(s) of identity verification:			IR methods

Table 2. Test-strains and their characterization.

Name/description	Characteristic	Source	Article-No.
<i>Salmonella typhimurium</i> TA1535	His G46, rfa uvrB, pKM-101, R-BPS	ATCC	29629
<i>Salmonella typhimurium</i> TA1537	His C3076, rfa uvrB, R-FS	ATCC	29630
<i>Salmonella typhimurium</i> TA98	His D3052, rfa uvrB, pKM-101, R-FS	NBRC	14193
<i>Salmonella typhimurium</i> TA100	His G46, rfa uvrB, R-BPS	NBRC	14194
<i>Escherichia coli</i>	WP2 uvrA, trp	ATCC	49979

Abbreviations: His G46, His C3076, His D3052 - frameshift missense mutations, rfa uvrB, uvrA - increased sensitivity to chemicals that interfere with base pair substitution, pKM-101 - mutagenesis-enhancing resistance transfer plasmid (R plasmid).

Table 3. Positive controls and their characterization.

Chemical and CAS No.	Manufacturer	Lot #	Purity
Benzo(a)pyrene (CAS no. 50-32-8)	Sigma-Aldrich	SLBP3558V	≥96%
2-Aminoanthracene (CAS no. 613-13-8)	Sigma-Aldrich	STBD3302V	96%
Sodium azide (CAS no. 26628-22-8)	Sigma-Aldrich	BCBQ6198V	≥99.5%
9-Fluorenone (CAS no. 486-25-9)	Sigma-Aldrich	STBC8059V	98%
Methylmethanesulfonate (CAS no. 66-27-3)	Sigma-Aldrich	MKBL6789V	99%

Abbreviations: 2-AA - 2-aminoanthracene, B(a)p - benzopyrene, SA - Sodium azide, TNF - fluorenone, MMS - methylmethanesulfonate.

EXPERIMENTAL

Materials

Test Item and its characteristics are presented in Table 1.

Preparation of $2(C_6H_{15}N_2O_2)+2I_3-H_2O$ iodine co-ordination complex stock solution

Maximal concentration of test item substance was 5000 µg/plate and had been defined on the basis of recommendations of manual N° 471 OECD (The Organisation for Economic Co-operation and Development) Test Guideline (13). Other 4 concentrations of test item for cytotoxicity assay were received by $\sqrt{10}$ -fold dilution of maximal concentration. All working solutions of the test item were prepared by dilution of a primary test substance in a sterile PBS solution.

A stock solution was prepared by dissolving 0.5 g of the test item 10.0 mL of sterile PBS.

Test strains

Genetic toxicology standard strains of gram-negative rods *Escherichia coli* and *Salmonella typhimurium* were used as a test-systems according to OECD N° 471 "Bacterial Reverse Mutation Test" (Table 2).

Tester strains used were *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP2uvrA. *Salmonella typhimurium* TA1535, TA1537, and *Escherichia coli* WP2uvrA strains were received from American Type Culture Collection (ATCC, USA). *Salmonella typhimurium* TA98 and TA100 were received from the Biological Resource Center (NBRC) National Institute of Technology and Evaluation (NITE, Japan). Culture stocks were stored below -80°C until used. Recultivation of tester strain was performed

by adding 5 mL of Luria-Bertani broth to the permanent culture and incubating for 18-24 h at $37 \pm 1^\circ\text{C}$ at 100 rpm, to yield 1.5×10^8 cells/mL. Genotype confirmation of test-strains as histidine requirement, rfa mutation, tryptophan requirement, uvrA mutation, R-factor, and spontaneous reversion tests were performed according to Maron and Ames (12).

Reference items

The following substances were used in the study as reference items (positive controls) (Table 3).

These substances are suggested as positive controls by the OECD Guideline N° 471 "Bacterial Reverse Mutation Test". Dimethyl sulfoxide (DMSO, Sigma-Aldrich), PBS (phosphate-buffer solution, Sigma-Aldrich) were used as solvents.

Metabolic activation system

The S9 fractions were obtained from Sigma-Aldrich (USA), and the mix for metabolic activation was prepared according to (14).

Cytotoxicity assay

The cytotoxicity test was performed to determine the highest concentration and was detected by observing the reduction in the number of reverting colonies, a clearing or diminution of the background lawn, or the degree of survival of treated cultures (12). Top agar for *Salmonella* was prepared by dissolving 6.0 g of bacteriological agar (Himedia, India), 5.0 g of NaCl (Sigma, USA), 10.5 mg of L-histidine (Sigma, USA) and 12.2 mg of biotin (Sigma, USA) in 1000 mL of distilled water and autoclaved for 15 min at 121°C . Top agar for *E. coli* was prepared by dissolving 7.0 g of bacteriological agar (Himedia, India), 6.0 g of NaCl (Sigma, USA), 10.2 mg of tryptophan (Sigma, USA) in 1000 mL of

distilled water and autoclaved for 15 min at 121°C. Overnight bacterial culture (0.1 mL) was added to 0.1 mL of test item (5000.0, 1581.0, 500.0, 158.0, 50.0 µg/plate) and 2 mL of top agar in the presence or absence of S9-mix. This was placed onto nutritive agar plates and incubated at 37 ± 1°C for 48 h.

Mutagenicity assay

The plate incorporation test was performed as previously described by Maron and Ames (12).

Briefly, 5 mL of nutrient broth and Luria-Bertani were inoculated by a single colony of *Salmonella typhimurium* and *E. coli* WP2 strains, respectively, and then the cultures were incubated at 37 ± 1°C overnight while shaking at 150 rpm.

Then, 0.1 mL of five concentrations of test item (500.0, 330.0, 158.0, 104.0 and 50.0 µg/plate), 0.1 mL of overnight bacterial culture (1×10⁸ cells/mL), 0.1 mL of positive or negative (phosphate-buffered saline, PBS) controls, and 0.5 mL of S9 mix or PBS, were mixed with 2 mL of top agar and poured on minimal glucose agar plates. After solidification of the top agar, the plates were incubated at 37 ± 1°C for 48 and 72 h. After incubating for 48 h at 37 ± 1°C, the number of his⁺ revertants for *S. typhimurium* strains and trp⁺ revertants for *E. coli*

WP2uvrA strains in the sample were compared to the negative control. All concentration samples were tested on triplicate plates.

Validation of the Test Procedure

The Test Procedure was validated before this study. The following parameters were validated (Table 4).

Statistical analysis

Colony counting was performed by colony counter by marking a dot over each colony on the plate. Data received from the experiments are presented as mean (M) ± standard deviation (SD) of three replicates. A student's *t*-test was performed for the statistical analysis of difference. The differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Cytotoxicity assay results

Ames test or *Salmonella*/microsome test is widely used in investigating the mutagenic activity of chemicals (12). If the test chemical have mutagenic or premutagenic effect on any test-strain of a bacterium, the number of histidine-independent

Table 4. Summary of validation results.

Parameter	Repeatability		
Characteristic	Analyzing 6 samples each at 100% of the test concentration		
Specification	CV ≤ 20%		
Results	For <i>E. coli</i> WP2 CV with MMS = 13.59%	Evaluation	Pass
	For TA1537 CV with TNF = 9.19%. For TA98 CV with TNF = 6.23%		Pass
	For TA1537 CV with B(a)p = 5.96%. For TA100 CV with B(a)p = 10.35%.		Pass
	For <i>E. coli</i> WP2 CV with 2AA = 8.0%. For TA1535 CV with 2AA = 6.14%. For TA98 CV with 2AA = 8.75%.		Pass
	For TA1535 CV with SA = 17.03%. For TA100 CV with SA = 4.36%.		Pass
Parameter	Specificity		
Characteristic	Three concentration without metabolic activation: SA - 0.75, 1.5, 3.0 µg/plate; TNF - 0.05, 0.1, 0.2 µg/plate; MMS - 1000, 2000, 4000 µL/plate. / Three concentration with metabolic activation 2AA for <i>Salmonella</i> - 1.25, 2.5, 5.0 µg/plate, for <i>E. coli</i> WP2 5.0, 10.0, 20.0 µg/plate.		
Specification	p ≤ 0.05		
Results	Without metabolic activation: SA for TA1535 p ≤ 0.007, TA100 p ≤ 0.0001; TNF for TA98 p ≤ 0.003, TA1537 p ≤ 0.005; MMS for <i>E. coli</i> WP2 p ≤ 0.01 With metabolic activation: 2AA for TA1535 p ≤ 0.0005, TA98 p ≤ 0.001; <i>E. coli</i> WP2 p ≤ 0.0001; B(a)p for TA1537 p ≤ 0.01, TA100 p ≤ 0.0001.	Evaluation	Pass

CV - coefficient of variation.

Table 5. Toxicity of test item in *S. typhimurium* TA98 strain in the absence or presence of a metabolic-activating system (S9).

Metabolic factivation system	Test substance	Conc. (µg/plate)	Number of colonies (Mean ± SD)
S9(-)	PBS	-	31 ± 2
	2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O	5000.0	-
		1581.0	-
		500.0	28 ± 3
		158.0	23 ± 2
		50.0	26 ± 4
	TNF	0.2	704 ± 21
S9(+)	PBS	-	145 ± 12
	2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O	5000.0	-
		1581.0	-
		500.0	144 ± 5
		158.0	136 ± 6
		50.0	147 ± 5
	2-AA	5.0	280 ± 30

Value are mean ± SD of three plates. Negative control (NC): PBS (100 µL/plate); positive control (PC): for TA98/-S9 – TNF (conc. 0.2 µg/plate); for TA98/+S9 – 2-AA (conc. 5.0 µg/plate).

Table 6. Toxicity of test item in *S. typhimurium* TA100 strain in the absence or presence of a metabolic-activating system (S9).

Metabolic factivation system	Test substance	Conc. (µg/plate)	Number of colonies (Mean ± SD)
S9(-)	PBS	-	106 ± 17
	2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O	5000.0	-
		1581.0	-
		500.0	106 ± 8
		158.0	91 ± 8
		50.0	105 ± 5
	NaN ₃	0.2	311 ± 30
S9(+)	PBS	-	194 ± 21
	2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O	5000.0	-
		1581.0	-
		500.0	193 ± 5
		158.0	190 ± 14
		50.0	198 ± 6
	B(a)p	5.0	526 ± 27

Value are mean ± SD of three plates. Negative control (NC): PBS (100 µL/plate); positive control (PC): for TA100 without metabolic activation (S9) - NaN₃ (conc. 3.0 µg/plate); for TA100 with metabolic activation (S9) - B(a)p (conc. 5.0 µg/plate).

colonies arising on those plates will be significantly greater than the corresponding control (negative) plates for that strain of bacteria (15). In this study it should be noted that two top concentrations – 5000.0 µg/plate and 1581.0 µg/plate showed a toxic effect on TA98, TA100 and *E. coli* WP2 test-strains,

having caused the complete death of revertant colonies and a background lawn compared to the negative controls (Table 5 – Table 7).

Application of 100 µL of 5000.0 µg/plate and 1581.0 µg/plate concentrations of the test substance to the *S. typhimurium* TA98 culture completely pre-

vented bacterial growth leading to zero colony count on the corresponding plates. At the higher dilutions (lower concentrations), bacterial growth was observable, both in the presence and absence of S9 rat liver fraction, so that the colony counts were approximately identical to the average number of colonies for the negative controls (Table 5).

Application of 100 µL of 5000.0 µg/plate and 1581.0 µg/plate concentrations of the test substance to the *S. typhimurium* TA100 culture completely prevented bacterial growth leading to zero colony count on the corresponding plates. At the higher dilutions (lower concentrations – 500.0, 158.0, 50.0 µg/plate), bacterial growth was observable, both in the presence and absence of S9 rat liver fraction, so that the colony counts were approximately identical to the average number of colonies for the negative controls (Table 6).

The iodine coordination complex was tested in the tester strain *E. coli* WP2 with concentrations of 50.0, 158.0, 500, 1581 and 5000 µg/plate in the absence and presence of S9 mix. The results are shown in Table 4. To determine the toxicity of iodine coordination complex the reduction of the bacterial background lawn, the increase in the size of the microcolonies and the reduction of the revertant colonies were examined. The tester strain *E. coli* WP2 treated with iodine coordination complex at the concentrations 1581.0 and 5000 µg/plate in the absence and presence of metabolic activation

revealed a background bacterial lawn inhibition when compared to vehicle control groups. Reduction of the bacterial background lawn and decrease in the number of revertants were observed over the concentrations 1581.0 to 5000 µg/plate.

Mutagenicity assay results

Therefore, we choose 500.0 µg/plate as the highest concentration for the test item. Total 5 concentrations of test item (500.0, 330.0, 158.0, 104.0 and 50.0 µg/plate) were tested (Table 8 – Table 9).

Apparently from Table 5, application of test item on *S. typhimurium* TA98 indicator test strain in concentration from 50.0 µg/plate to 500.0 µg/plate doesn't induce the frequency of reversions in comparison with positive control. The number of revertants of *S. typhimurium* TA100 test strain induced by test item in concentration from 50.0 to 500.0 µg/plate statistically didn't exceed a background of spontaneous mutations. The results obtained showed that all doses (500, 330, 158, 104 and 50 mg/plate) of the iodine coordination complex were not mutagenic for the TA98 and TA100 strains since there was no significant difference between the number of his + revertants of the treated groups and the solvent control group ($p > 0.05$). Furthermore, none of the tested strains reached a dose-response effect with the test item treatments. Thus, by obtained results, $2(C_6H_5O_3)_2 I_3H_2O$ – iodine coordination complex did not induce base-pair or frameshift mutations.

Table 7. Toxicity of test item in *E. coli* WP2 strain in the absence or presence of a metabolic-activating system (S9).

Metabolic factivation system	Test substance	Conc. (µg/plate)	Number of colonies (Mean ± SD)
S9(-)	PBS	-	79 ± 13
	$2(C_6H_5O_3)_2 I_3H_2O$	5000.0	-
		1581.0	-
		500.0	74 ± 5
		158.0	62 ± 6
		50.0	77 ± 11
	MMS	4000.0	521 ± 19
S9(+)	PBS	-	130 ± 6
	$2(C_6H_5O_3)_2 I_3H_2O$	5000.0	-
		1581.0	-
		500.0	137 ± 4
		158.0	128 ± 4
		50.0	129 ± 6
	2-AA	20.0	362 ± 15

Value are mean ± SD of three plates. Negative control (NC): PBS (100 µL/plate); positive control (PC): for *E. coli* WP2 without metabolic activation (S9) - MMS (conc. 4000.0 µg/plate); for *E. coli* WP2 with metabolic activation (S9) - 2-AA (conc. 20.0 µg/plate).

Table 8. Revertants in strains of *S. typhimurium* TA98 and TA100 treated with different concentrations of the test item in the absence or presence of a metabolic activation system (S9-mix).

Treatment and doses	Strains of <i>Salmonella</i>			
	TA98		TA100	
	S9(-)	S9(+)	S9(-)	S9(+)
2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O (500 µg/plate)	28 ± 3*	134 ± 5*	93 ± 13*	197 ± 13*
2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O (330 µg/plate)	31 ± 4*	134 ± 9*	94 ± 7*	186 ± 11*
2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O (158 µg/plate)	29 ± 3*	139 ± 7*	95 ± 10*	187 ± 14*
2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O (104 µg/plate)	28 ± 5*	140 ± 7*	99 ± 21*	183 ± 12*
2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O (50 µg/plate)	27 ± 3*	142 ± 14*	98 ± 3*	190 ± 21*
Positive control	702 ± 24	277 ± 24	316 ± 17	512 ± 23
Negative control	28 ± 2	142 ± 8	104 ± 12	193 ± 11

Value are mean ± SD of three plates. Negative control (NC): PBS (100 µL/plate); positive control (PC): for TA98 without metabolic activation (S9) – TNF (conc. 0.2 µg/plate); for TA98 with metabolic activation (S9) – 2-AA (conc. 5.0 µg/plate); positive control (PC): for TA100 without metabolic activation (S9) – NaN₃ (conc. 3.0 µg/plate); for TA100 with metabolic activation (S9) – B(a)p (conc. 5.0 µg/plate). *Significantly different from positive control plate group (p ≤ 0.05).

Table 9. Revertants in strains of *S. typhimurium* TA1535, TA1537 and *E. coli* WP2uvrA treated with different concentrations of the test item in the absence or presence of a metabolic activation system (S9-mix).

Treatment and doses	Strains of <i>Salmonella</i> and <i>E. coli</i> WP2					
	TA1535		TA1537		<i>E. coli</i> WP2	
	S9(-)	S9(+)	S9(-)	S9(+)	S9(-)	S9(+)
2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O (500 µg/plate)	19 ± 2*	50 ± 2*	29 ± 7*	125 ± 8*	62 ± 5*	132 ± 5*
2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O (330 µg/plate)	25 ± 1*	52 ± 3*	28 ± 3*	125 ± 8*	62 ± 8*	127 ± 10*
2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O (158 µg/plate)	21 ± 2*	53 ± 5*	26 ± 3*	132 ± 7*	58 ± 7*	134 ± 6*
2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O (104 µg/plate)	23 ± 2*	50 ± 7*	30 ± 3*	127 ± 10*	61 ± 6*	133 ± 5*
2(C ₆ H ₁₅ O ₅) 2I ₃ H ₂ O (50 µg/plate)	22 ± 3*	52 ± 7*	27 ± 6*	129 ± 3*	67 ± 7*	133 ± 10*
Positive control	171 ± 6	271 ± 17	289 ± 18	232 ± 7	529 ± 12	397 ± 36
Negative control	22 ± 2	55 ± 2	25 ± 9	130 ± 10	62 ± 6	132 ± 4

Value are mean ± SD of three plates. Negative control (NC): PBS (100 µL/plate). Positive controls (PC): for TA1535 with metabolic activation (S9) – NaN₃ (3.0 µg/plate); for TA1535 with metabolic activation (S9) – 2-AA (conc. 5.0 µg/plate); for TA1537 without metabolic activation (S9) – TNF (conc. 0.2 µg/plate); for TA1537 with metabolic activation (S9) – B(a)p (conc. 5.0 µg/plate); for *E. coli* WP2 without metabolic activation (S9) – MMS (conc. 4000.0 µg/plate); for *E. coli* WP2 with metabolic activation (S9) – 2-AA (conc. 20.0 µg/plate). * Significantly different from positive control plate group (p ≤ 0.05).

Based on the data on the number of revertant colonies observed during the dose range-finding study was considered for the main mutagenicity study. There was no reduction of the bacterial background lawn and no biologically relevant decrease

in the number of revertants at any of the concentration tested in TA1535 and TA1537 tester strains in the absence and presence of metabolic activation. In the main mutagenicity assay, no increase in the number of revertants was observed upon treatment

with iodine coordination complex under all conditions tested.

$2(C_6H_{15}O_5)2I_3H_2O$ did not exhibit mutagenic effects on *S. typhimurium* and *E. coli* WP2 strains. Significant differences were found between the revertant colonies compared between positive controls and test item concentrations ($p \leq 0.05$).

The aim of this study was to evaluate the mutagenic effects induced by $2(C_6H_{15}N_2O_2) + 2I_3 \cdot H_2O$ iodine coordination complex using the popular Ames test. This investigation was carried out using the *S. typhimurium* and *E. coli* WP2 test strains. This is an available and reliable biochemical technique for evaluating the mutagenic potential of biomaterials. (14, 16, 17, 18, 19). The preliminary toxicity assay should be performed in the absence and presence of a metabolic activation system which is to be used in the final mutagenicity assay.

When histidine-dependent bacteria are grown on a glucose-minimal (GM) agar plate containing a trace amount of histidine, only those cells that revert to histidine-independence (His⁺ for *Salmonella*) or tryptophan-independence (trp⁺ for *E. coli*) state are able to form colonies. The small amount of histidine or tryptophan allows all the plated bacteria to undergo a few cell divisions; in many cases, this growth is crucial for mutagenesis to occur. The His⁺ and trp⁺ revertants are easily scored as colonies against the slight background growth. The number of spontaneously induced revertant colonies is relatively constant for each strain. However, when a mutagen is added to the plate, the number of revertant colonies per plate is increased (17).

In this experimental study $2(C_6H_{15}O_5) 2I_3H_2O$ iodine-containing complex was tested in a bacterial test system that is able to register gene mutations with and without metabolic activation system. In all test-strains applied in the present study absolutely negative results were obtained.

It is noteworthy that the frequencies of spontaneous gene mutations in all bacterial strains as well as those induced by positive controls were within the ranges described by other investigators (20-22). The lack of genotoxic potential of the iodine-containing complex in this test is not surprising. The main components of the compound, iodine and amino acids are not genotoxic. From literature data concerning possible genotoxicity and carcinogenicity of iodine and its derivatives and did not find any evidence of genotoxic activity of these compounds (23). Moreover, Chan et al. used the Ames/Salmonella assay to determine the antimutagenic effect of heated lysine-fructose solution (24).

Interesting, that povidone-iodine, widely used representative of this class of iodine preparations, is non-genotoxic in several *in vitro* mutagenicity tests in bacteria and mammalian cells as well in mice bone marrow MN assay (25-27). These findings support the results obtained in this study.

Genotoxicity tests are useful tools in the investigation of possible harmful effects of drugs (i.e. mutagenicity, carcinogenicity). Brambilla et al. reviewed the literature data regarding DNA-damaging activity of 146 pharmaceuticals widely used in modern medicine and showed 63.1% concordance in the case of DNA strand breaks and carcinogenicity of the drugs (28). The same authors reviewed the literature data on genotoxicity of 48 antibacterial, antiviral, antimalarial and antifungal drugs (29). They found that concordance between carcinogenicity and positive genotoxic effects for these widely used in clinical practice drugs in *in vitro* and *in vivo* cytogenetic tests, bacterial mutagenicity tests and DNA damaging activity was 70%, 50%, 50%, and 43%, respectively.

In conclusion, $2(C_6H_{15}O_5) 2I_3H_2O$ iodine-containing complex showed a negative result in the bacterial reverse mutation test, indicating that it is not mutagenic. Mutagenic effect of the test item in concentrations from 0.05 up to 0.5 mg/plate with and without metabolic activation was not revealed in the bacterial reverse mutation test in *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 test strains. However, further experiments are needed to study the genotoxicity potential of $2(C_6H_{15}O_5) 2I_3H_2O$ iodine-containing complex.

CONCLUSION

The results both with and without metabolic activation were negative for all strains. It should be noted that two top concentrations – 5.0 mg/plate and 1.581 mg/plate showed a toxic effect on all test-strains, having caused the complete death of revertant colonies and a background lawn. The results of the Bacterial Reverse Mutation Test indicate that Test Item – $2(C_6H_{15}O_5)2I_3H_2O$ iodine-containing complex did not induce significant increases in the frequency of revertants for all test strains in the presence and absence of metabolic activation in comparison to the positive controls. Therefore, under the conditions of this test and according to the criteria set for the evaluation of the test results, the Test Item did not show mutagenic activity in the *S. typhimurium* and *E. coli* plate incorporation assay.

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Conflict of interest

The authors declare no conflicts of interest.

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