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Studies of mutagenic activity of fluorescent DNA-sensitive monomethinecyanine and carbocyanine dyes in Ames test

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Summary. Cyanine dyes are widely used as fluorescent probes for visualization of nucleic acids in electrophoretic gels. Unfortunately, some of these dyes are known to have mutagenic and carcinogenic activity.

Here, four cyanine dyes **D-6**, **D-9**, **CPent V** and **CCyan 2-O**, proposed before as fluorescent dyes for DNA visualization in gels [1], were evaluated for mutagenicity in the *Salmonella typhimurium* assay (Ames assay) and cytotoxicity. The most sensitive of the studied dyes, mesosubstituted carbocyanine **CCyan 2-O**, was selected for further examination in acute toxicity assay on laboratory animals.

Results indicated that according to the '2-fold rule' of assessing mutagenicity in the Ames assay monomethinecyanines **D-6** and **D-9** are not mutagenic, and carbocyanines **CCyan2-O** and **CPent-V** appeared to be weak mutagens. Mutagenic activity of carbocyanines was up to two times higher as compared to monomethine dyes.

Carbocyanines **CCyan2-O** and **CPent-V** demonstrated higher cytotoxicity, being tested against *Salmonella* cells strain TA98, than did monomethinecyanines **D-6** and **D-9**. Acute toxicity test indicates that the oral LD50 of **CCyan 2-O** for albino mice and rats are equal to 53.0 and 56.5 mg/kg, respectively. The lowest dose causing lethality LDLO [2] for both species of rodents amounted to 20 mg/kg.

Key words: cyanine dyes, mutagenicity, acute toxicity, LD50, *Salmonella* mutation assay.

Introduction. The mutagenicity and toxicity testing of commercially available dyes used in research investigations and clinical diagnostic is crucial for accurate predicting of health risks for consumers and workers exposed to dyes [3]. Unfortunately, these data are often lacking. For example, the phenanthridium dye Ethidium bromide that is known to be the most often used dye in molecular biology and genetic engineering experiments for DNA detection in electrophoretic gels [4, 5], appeared to have undesirable mutagenic and carcinogenic activity [6], evaluated in the *Salmonella*/mammalian microsome reverse mutation assay [7]. The point is that Ethidium bromide could interfere with nucleic acids synthesis that results in replication inhibition and causes frameshift mutations in bacteria [6, 8]. Cyanine dimer dyes TOTO-1 and YOYO-3 besides the ability to intercalate into dsDNA molecule also appeared to have complex binding modes, obviously involving interactions with sites in the major or minor grooves and/or with the phosphate backbone of DNA molecules [7].

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Such properties could point to the possibility that above mentioned cyanine dyes are mutagenic, however, we couldn't find any reports about their mutagenicity test results.

For today cyanine dyes are successfully used in research investigations and clinical diagnostics as most sensitive fluorescent probes for nucleic acids detection [9]. Earlier we proposed thia(pyrido-4)monomethinecyanines [10] and mesosubstituted carbocyanines as efficient dyes for DNA determination in solution. In previous paper we studied applicability of the series of monomethine- and carbocyanines as stains for DNA visualization in agarose gels [1]. Upon UV-transillumination the methyl-mesosubstituted carbocyanine **CCyan 2-O** was shown to be of the same sensitivity as commonly used stain Ethidium bromide. In order to design safe and sensitive fluorescent DNA probes we determined mutagenic potential and cytotoxic activity of monomethines **D-6**, **D-9** and carbocyanines **CPent V**, **CCyan 2-O**. Among the series of dyes they were selected as most successful stains for post-electrophoretic visualization.

The aim of present study was to evaluate and compare mutagenicity of these selected cyanine dyes in the plate incorporation assay with *Salmonella typhimurium* strains TA98 and TA100. Mutagenicity assays were performed both in the presence and absence of a rat liver microsome preparation (S9) in order to define the mutagenic potential not only of the studied cyanines themselves, but also of their metabolites. We tested cytotoxity of the **D-6**, **D-9**, **CPent V** and **CCyan 2-O** cyanine dyes with tester strain TA98 (*Salmonella* cells of TA98 strain are defective in excision-repair system and therefore are very sensitive to DNA damaging factors). For the most sensitive dye of the series, carbocyanine **CCyan 2-O**, the acute toxicity on albino mice and rats was evaluated as well.

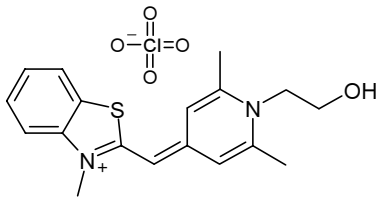
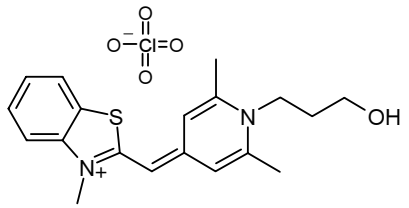
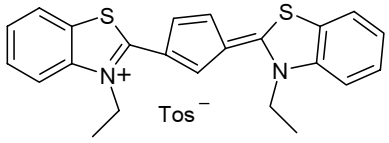
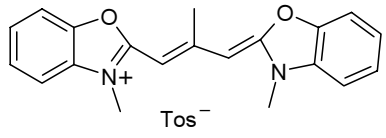
Materials and methods. ***Chemicals.*** Cyanine dyes **D-6** and **D-9** were synthesized as described by Yarmoluk et al [11]. Carbocyanines **CPent V** and **CCyan 2-O** were kindly provided by Dr. Yu. Slominskii (Institute of Organic Chemistry of the NAS of Ukraine, Kyiv, Ukraine) [12, 13]. Chemical structures of studied cyanines are presented in Table 1. 5-ethyl-5-phenylbarbituric acid (phenobarbital), glucoso-6-phosphate (G-6-P) and nicotinamide adenindinucleotidphosphate (NADP) were from Sigma (St. Louis, USA). Aflatoxin (AFBi), 4-nitro-O-phenylenediamine (NPD), ethylnitrosourea (ENU), thiobarbituric acid and inorganic salts for bacterial assays were purchased from Merck (Darmstadt, Germany). Bacterial media were from Difco (Detroit, USA).

Laboratory animals. Male albino outbred mice weighting in average 20 g and albino outbred rats weighting in average 150 g (used for acute toxicity testing) and 200 g (used for micronucleus assay) were obtained from vivarium of the Institute of Microbiology and Virology of the NAS of Ukraine (Kyiv, Ukraine); age of the animals ranged from 8 to 12 weeks. The rodents were fed laboratory chow and water and maintained in an air-conditioned environment (23±1 °C, 50±5 % humidity) in a 12 h light/dark cycle.

Microsomal enzyme preparation method. Three male albino outbred rats (average weight 200 g) were treated by intraperitoneal adminis-

Table 1

Chemical structures of studied cyanine dyes

Name	Structure
D-9	
D-6	
CPent-V	
CCyan 2-O	

tration of 0.5 ml of phenobarbital (80 mg/kg) during three days for induction of microsomal enzymes in liver. The animals were sacrificed by decapitation in 24 h after last treatment. Liver enzyme homogenate (fraction S9) was prepared according to [14], using phenobarbital as inductor of microsomal enzymes in rat liver. Mixture S9 was prepared before using and contained in 1 ml: fraction S9 0.1 ml, 8 μM $MgCl_2$, 33 μM KC1, 5 μM G-6-P, 4 μM NADP and 100 μM Na-phosphate buffer, pH 7.4.

Salmonella typhimurium reverse mutation assay. The plate incorporation assay was carried out with *Salmonella typhimurium* tester strains TA98 and TA100 in His^+ reversion test as described by Maron and Ames [14] and following EU Commission directive 2000/32/EC, B.13/14 [15].

Salmonella typhimurium strains TA98 and TA100 were obtained from Dr. A. Dugan (Institute of Hygiene and Medical Ecology of the AMS of Ukraine, Kyiv, Ukraine). These strains are commonly used for screening on mutagenicity because they indicate both frameshift (TA98) and base-pair (TA100) mutations. Both cultures were purified from spontaneous His^+ revertants before use.

For all of the dyes five doses (20, 40, 60, 80 and 100 μg/per plate) were tested in both the presence and absence of S9 mixture. Positive controls for TA98 were 20 μg/per plate of 4-nitro-O-phenylenediamine (NPD) (1512 revertants) without S9 and 2 μg/per plate of aflatoxin (AFB_1) (458 revertants) with metabolic activation. Positive controls for TA100 were 400 μg/per plate of ethylnitrosourea (ENU) (306 revertants) in experiments without S9 mixture and 2 μg/per plate of AFB_1 (582 revertants) with metabolic activation. Each dose was plated in triplicate. Following incubation at 37±2 °C for 48±8 h, revertant colonies were counted. A sample was evaluated as mutagenic, if there was more than a doubling of revertant colonies per plate in comparison to the control in at least one strain with or without the metabolic activation system and/or if a dose-dependent increase occurred over the range tested [16].

Cytotoxicity assay. The cytotoxicity of the studied dyes was tested against Salmonella cells strain TA98, obtained from Dr. A. Dugan (Institute of Hygiene and Medical Ecology of the AMS of Ukraine, Kyiv, Ukraine). Cells of this tester strain are defective in excision-repair system and therefore are very sensitive to DNA damaging factors, so TA98 was chosen for the dyes cytotoxicity evaluation.

For all of the dyes five doses (5, 10, 20, 30 and 40 μg/per plate) were tested and after incubation at 37±2 °C for 48±8 h the number of colonies in each plate was counted. For each dye vehicle control was performed as well. The results represent percent of survived colonies in regard to number of colonies in corresponding vehicle control.

Study of acute toxicity of CCyan 2-O. In previous studies [1] mesosubstituted carbocyanine **CCyan 2-O** was shown as the most sensitive stain for post-electrophoretic DNA visualization. Taking into account comparatively week mutagenicity of this carbocyaniane, it was supposed to be a promising DNA probe, so this dye was selected for further examination in acute toxicity assay.

Male albino outbred mice and outbred rats, used for this experiment, were kept in vivarium (Institute of Microbiology and Virology of the NAS of Ukraine, Kyiv, Ukraine) under standard conditions. 60 male albino outbred mice (average weight 20 g) were divided into six groups of ten animals in each group, and 60 albino outbred rats (average weight 150 g) were divided into groups in the same manner. Different doses of cyanine dye **CCyan 2-O** (10, 20, 40, 60, 80 and 100 mg/kg) dissolved in dimethylsulfoxide and mixed with starch paste were orally administrated to different groups of mice and rats. Dose levels were selected following Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity [17]. Experimental observation of animals continued during one week. Calculation of oral LD50 index was carried out according to Kerber formula [18]:

$$\mathbf{LD50 = LD100 - \Sigma (z \times d)/m}$$

where **z** is a half of sum of animal quantity died from two next doses;

d is the interval between two next doses;

m is the number of animals.

Results. ***Salmonella typhimurium reverse mutation assay.*** Study of monomethinecyanine dyes **D-6** and **D-9** and carbocyanines **CCyan 2-O**

Table 2

Mutagenicity assay results for +1 frameshift indicator strain TA98 and base-substitution indicator strain TA100[a]

Dye	Dose (μg/per plate)	TA98		TA100	
		no S9 mix	in S9 mix presence	no S9 mix	in S9 mix presence
D-6	Vehicle control	40 ± 1	40 ± 1	191 ± 6	191 ± 6
	5	48 ± 3	32 ± 3	216 ± 11	169 ± 7
	10	52 ± 4	41 ± 2	240 ± 8	150 ± 5
	20	50 ± 3	40 ± 1	210 ± 7	135 ± 8
	30	46 ± 3	36 ± 2	180 ± 7	120 ± 5
	50	42 ± 3	30 ± 1	114 ± 4	81 ± 2
	100	39 ± 1	28 ± 3	98 ± 5	65 ± 2
	Positive control[b]	1512 ± 7	458 ± 17	306 ± 11	582 ± 9
D-9	Vehicle control	40 ± 1	40 ± 1	191 ± 6	191 ± 6
	5	62 ± 2	34 ± 2	237 ± 10	181 ± 5
	10	65 ± 3	44 ± 3	280 ± 13	175 ± 11
	20	59 ± 3	45 ± 3	240 ± 8	150 ± 6
	30	57 ± 3	48 ± 3	200 ± 7	125 ± 4
	50	60 ± 2	52 ± 8	186 ± 11	116 ± 5
	100	63 ± 6	53 ± 2	177 ± 7	110 ± 5
	Positive control	1512 ± 7	458 ± 17	306 ± 11	582 ± 9
CCyan2-O	Vehicle control	40 ± 1	40 ± 1	191 ± 6	191 ± 6
	5	118 ± 4	160 ± 6	400 ± 13	550 ± 20
	10	130 ± 4	165 ± 6	620 ± 20	764 ± 24
	20	24 ± 3	48 ± 1	509 ± 15	630 ± 23
	30	15 ± 3	32 ± 2	408 ± 13	506 ± 20
	50	2 ± 1	20 ± 3	212 ± 6	262 ± 6
	100	2 ± 1	18 ± 3	64 ± 4	80 ± 3
	Positive control	1512 ± 7	458 ± 17	306 ± 11	582 ± 9
CPent-V	Vehicle control	40 ± 1	40 ± 1	191 ± 6	191 ± 6
	5	85 ± 3	100 ± 4	142 ± 8	190 ± 11
	10	104 ± 4	115 ± 4	97 ± 6	194 ± 8
	20	14 ± 2	34 ± 1	69 ± 7	146 ± 6
	30	5 ± 2	20 ± 1	46 ± 2	98 ± 4
	50	4 ± 2	10 ± 1	24 ± 3	68 ± 3
	100	4 ± 2	8 ± 4	7 ± 1	14 ± 3
	Positive control	1512 ± 7	458 ± 17	306 ± 11	582 ± 9

[a]*Mean number of revertants per plate ± standard deviation, for experiments performed in triplicate.*
[b]*Positive controls for TA98 strain were 20 μg/per plate of 4-nitro-O-phenylenediamine (NPD) in the absence of S9 and 2 мg/per plate of aflatoxin (AFB_1) in the presence of metabolic activation; in case of TA100 strain positive controls were 400 μg/per plate of ethylnitrosourea (ENU) in experiments without S9 mixture and 2 μg/per plate of AFB_1 in S9 presence.*

and **CPent-V** mutagenicity was carried out on *Salmonella* cells strains TA98 and TA100. The results of these independent mutagenicity assays, presented as mean revertants per plate ± standard deviation, are shown in Table 2.

Frameshift activity. *Monomethine cyanines* ***D-6*** *and* ***D-9***. Small increase in His^+ revertants frequencies per plate induced by thia(pyrido-4)monomethinecyanines were observed using +1 frameshift mutation-indicating tester strain TA98 in the absence of S9 mixture (maximum increases of 1.3 and 1.63-fold in cases of **D-6** and **D-9** dyes respectively). No increases induced by monomethinecyanine **D-6** were observed with

TA98 strain in the presence of metabolic activation. However, **D-9** dye induces small increase in the frequencies of His^+ revertants (maximum of 1.33 fold) in the S9 mix presence (Table 2).

*Carbocyanines **CCyan 2-O** and **CPent-V***. Revertant frequencies increases induced by carbocyanines **CCyan 2-O** and **CPent-V** observed with the TA98 tester strain were approximately from two to four times higher as compared to monomethines **D-6** and **D-9**. In such a way, maximum increases of 3.25- and 2.6-fold in cases of **CCyan 2-O** and **CPent-V** respectively were noticed with this tester strain in the absence of metabolic activation. Increases in revertants frequencies in the presence of S9 mix were equal to 4.13-fold for mesosubstituted benzoxazole dye **CCyan 2-O** and 2.88-fold for **CPent-V** carbocyanine (Table 2).

***Base-substitution activity. D-6** and **D-9** thia(pyrido-4)monomethinecyanines*. Small increases in revertant frequencies induced by monomethinecyanines were observed with the base-substitution tester strain TA100 (increases of 1.26- and 1.47-fold for **D-6** and **D-9** correspondingly). No increases were noticed with tester strain TA100 in the presence of metabolic activation (Table 2).

***CCyan 2-O** and **CPent-V** carbocyanine dyes*. No increases in revertant frequencies were observed for carbocyanine dye **CPent-V** in either the presence or absence of metabolic activation. As for the carbocyanine dye **CCyan2-O** increases in revertant frequencies were admitted with tester strain TA100 in both the presence (2.88-fold) and absence (2.6-fold) of S9 mix (Table 2).

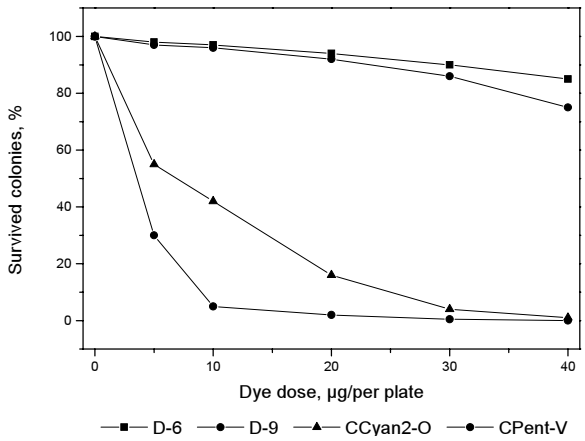


Fig. 1. Lethal effect of cyanine dyes on Salmonella typhimurium TA98 cells.

Cytotoxicity of cyanine dyes studied with Salmonella cells strain TA98. The cytotoxicity of the studied dyes was tested against *Salmonella* cells strain TA98, and the results are presented in Figure 1. Cells of this tester strain are defective in excision-repair system and therefore are very sensitive to DNA damaging factors.

In the bacterial strain tested, carbocyanines **CCyan2-O** and **CPent-V** demonstrated higher cytotoxicity than did monomethinecyanines **D-6** and **D-9**. The doses of 10 μg/per plate of dyes **D-6** and **D-9** induced death of only 3 % and 4 % of cells, respectively. For the comparison, studied carbocyanines in the same doses induced death of more than half *Salmonella* cells (about 58 % and 95 % for **CCyan2-O** and **CPent-V** accordingly). The maximal tested dyes doses (40 μg/per plate) in case of carbocyanines **CCyan2-O** and **CPent-V** provoked death of 99 % and 100 % cells respectively, while thia(pyrido-

Table 3

*Acute toxicity of cyanine dye **CCyan 2-O** for mice*

Dose, mg/kg	Number of died mice/total number	**z**	**d**	**z × d**
100	10/10	–	–	–
80	8/10	9.0	20	180
60	5/10	6.5	20	130
40	4/10	4.5	20	90
20	2/10	3.0	20	60
10	0/10	1.0	10	10

$\Sigma(\mathbf{z} \times \mathbf{d})=470$

z — half of sum of animal quantity died from two next doses, d — interval between two next doses.

Table 4

*Acute toxicity of cyanine dye **CCyan 2-O** for rats*

Dose, mg/kg	Number of died rats/total number	**z**	**d**	**z × d**
100	10/10	–	–	–
80	8/10	9.0	20	180
60	5/10	6.5	20	130
40	3/10	4.0	20	80
20	1/10	2.0	20	40
10	0/10	0.5	10	5

$\Sigma(\mathbf{z} \times \mathbf{d})=435$

z — half of sum of animal quantity died from two next doses, d — interval between two next doses.

4)monomethinecyanines **D-6** and **D-9** induced death of merely 15 % and 25 % of the *Salmonella* cells correspondingly.

Acute toxicity of cyanine dye CCyan 2-O. The results of experiments on acute toxicity of the mesosubstituted benzoxazole dye **CCyan 2-O** are presented in Tables 3 and 4. The index LD50 (dose of a drug to induce 50 % death of experimental animals) was calculated after Kerber formula.

No death of mice and rats treated with 10 mg/kg of **CCyan 2-O** were observed within seven days. In dose of 100 mg/kg this cyanine caused lethal outcome of all mice and rats within corresponding groups. The results indicate that the oral LD50 of **CCyan 2-O** for albino mice and rats are equal to 53.0 and 56.5 mg/kg respectively. The lowest dose causing lethality LDLO [2] for both species of rodents amounted to 20 mg/kg.

Discussion. The results of this study suggest that fluorescent monomethinecyanine dyes **D-6** and **D-9** are not mutagenic, and carbocyanines **CCyan 2-O** and **CPent V** turned to be weak mutagens in reversion mutation assay with *Salmonella typhimurium* tester strains TA98 and TA100.

The values of the peak mutagenic response exhibited by the studied monomethinecyanine dyes didn't exceed 1.63 (for **D-9** dye with tester strain TA98 in the absence of S9 mix). The higher increase in mutagenicity was observed for carbocyanines: 4.13- and 2.88-fold for **CCyan2-O** and **CPent-V** dyes respectively (in both cases with tester strain TA98 in the S9 mix presence). If we apply the '2-fold rule' of assessing mutagenicity in the Ames assay [19], our results show that **D-6** and **D-9** dyes are not mutagenic, and carbocyanines **CCyan2-O** and **CPent-V** appeared to be weak mutagens. For comparison, Singer et al. [7] shown Ethidium bromide to induce up to 70.9-fold increase in revertant frequencies with +1 frameshift indicator strain TA98 in the presence of S9 mix, whereas for carbocyanine **CCyan 2-O**, that had the highest mutagenicity among the studied dyes, this increase was only 4.13-fold.

The cytotoxicity of the studied dyes was tested against *Salmonella* strain TA98, cells of which are defective in excision-repair system and therefore are very sensitive to DNA damaging factors. In the bacterial strain tested, carbocyanines **CCyan2-O** and **CPent-V** demonstrated higher cytotoxicity, than monomethinecyanines **D-6** and **D-9**. The lowest dose (5 μg/per plate) of dye **D-6** induced death only of 2 % cells and **D-9** — 3 %, whereas **CCyan2-O** and **CPent-V** in the same dose provoked respectively 45 % and 70 % cell death. In the dyes dose of 10 μg/per plate monomethines caused death of less than 5 % *Salmonella* cells, and in the maximum dose (40 μg/per plate) — less than 50 %. Carbocyanine dye **CCyan 2-O** in dose of 10 μg/per plate induced death of 58 % cells, and after incubation of the cells in presence of 30 μg/per plate more than 90 % of the cells died. Carbocyanine dye **CPent-V** induced death of more than 90 % cells already in dose of 10 μg/per plate. Thus it was shown that studied monomethinecyanines are much less cytotoxic than carbocyanines and in final tested dose (40 μg/per plate) induced death only of 15 % and 25 % (**D-6** and **D-9** respectively) *Salmonella* cells. Among studied carbocyanines, dye **CPent-V** appeared to be more cyto-

toxic than **CCyan2-O**, but in dose of 40 μg/per plate both of the dyes provoked death of almost all of the cells.

Carbocyanine **CCyan 2-O** appeared to be a promising probe for DNA detection, and its sensitivity is close to that of commonly used Ethidium bromide [1], but its mutagenic potential is about 17-times lower as compared to Ethidium bromide. Therefore, it seemed to us it was relevant to study potential toxicity of carbocyanine **CCyan 2-O** in the acute toxicity experiment.

The acute toxicity of mesosubstituted benzoxazole dye **CCyan 2-O** was studied on outbred mice and rats. Results of the present study indicated that for dye **CCyan 2-O** given orally to mice and rats oral LD50 were equal to 53.0 and 56.5 mg/kg for albino mice and rats respectively. According to the Hodge and Sterner (1956) toxicity scale [19], this mesosubstituted benzoxazole dye is moderately toxic. The oral LDLO for both species of animals was 20 mg/kg.

Conclusions

1. Monomethinecyanines **D-6**, **D-9** and carbocyanines **CCyan2-O**, **CPent-V** that are promising as fluorescent stains for DNA detection were studied for their mutagenicity and cytotoxicity.

2. Monomethinecyanines **D-6** and **D-9** were considered to be non-mutagenic and carbocyanine dyes **CCyan2-O** and **CPent-V** appeared to be weak mutagens, according to the '2-fold rule' of assessing mutagenicity in the Ames assay. Mutagenic activity of carbocyanines was up to two times higher as compared to monomethine dyes.

3. Carbocyanines **CCyan2-O** and **CPent-V** demonstrated higher cytotoxicity, tested against *Salmonella* cells strain TA98, than did monomethinecyanines **D-6** and **D-9**.

4. Acute toxicity test indicates that the oral LD50 of carbocyanine **CCyan 2-O** for albino mice and rats are equal to 53.0 and 56.5 mg/kg, respectively. The lowest dose causing lethality LDLO for both species of rodents amounted to 20 mg/kg.

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Вивчення мутагенної активності флуоресцентних ДНК-чутливих монометинціанінових та карбоціанінових барвників у тесті Еймса

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Резюме. Ціанінові барвники широко використовуються як флуоресцентні зонди для візуалізації нуклеїнових кислот в електрофоретичних гелях. На жаль, деяким з таких барвників притаманна мутагенна та канцерогенна активність. У даній роботі досліджено мутагенну активність у тесті Еймса за використання штамів TA98 і TA100 *Salmonella typhimurium* та цитотоксичність чотирьох ціанінових барвників **D-6**, **D-9**, **CPent V** і **CCyan 2-O**, які, як засвідчили попередні дослідження, є чутливими флуоресцентними зондами для візуалізації ДНК у гелі [1].

Найбільш чутливий серед досліджуваних сполук мезо-заміщений карбоціаніновий барвник **CCyan 2-O** був обраний для подальшого тестування його токсичної активності на лабораторних тваринах.

Продемонстровано, що монометинціанінові барвники **D-6** та **D-9** є немутагенними, а карбоціанінам **CCyan2-O** та **CPent-V** притаманна слабка мутагенна активність.

Карбоціанінові барвники **CCyan2-O** та **CPent-V** виявилися більш цитотоксичними проти клітин *Salmonella* штаму TA98 у порівнянні з монометинціанінами **D-6** і **D-9**. У дослідженнях гострої токсичності з'ясовано, що оральна середня летальна доза LD50 барвника **CCyan2-O** для мишей та щурів становила 53,0 та 56,5 мг/кг відповідно. Найнижча летальна доза LDLO [2] для обох видів гризунів — 20 мг/кг.

Ключові слова: ціанінові барвники, мутагенність, гостра токсичність, LD50, тест Еймса.

References

1. *Matselyukh B.P., Yarmoluk S.M., Matselyukh A.B., Kovalska V.B., Kocheshev I.O., Kryvorotenko D.V., Lukashov S.S.* Interaction of cyanine dyes with nucleic acids: XXI. Using of polymethine cyanine dyes for the visualization of DNA in agarose gels // J. Biochem. Biophys. Methods. — 2003. — Vol. 57. — P. 35—43.

2. CCOHS, Canadian Centre for Occupational Health and Safety. OHS Answers: What is an LD50 and LC50. [June 2005].

3. *Wollin K.M., Gorlitz B.D.* Comparison of genotoxicity of textile dyestuffs in Salmonella mutagenicity assay, in vitro micronucleus assay, and single cell gel/comet assay // J. Environ. Pathol. Toxicol. Oncol. — 2004. — Vol. 23. — P. 267—278.

4. *Maniatis T., Fritsch E.F., Sambrook J.* Molecular Cloning: A laboratory Manual, Cold Spring Harbor, Cold Spring Harbor Laboratory Press, 1982.

5. *LePecq J.B., Paoletti A.* A new fluorometric method for RNA and DNA determination // Anal. Biochem. — 1966. — Vol. 17. — P. 100—107.

6. *McCann J., Choi E., Yamasaki E., Ames B.N.* Detection of carcinogenes as mutagens in the Salmonella/microsome test: assay of 300 chemicals // Proc. Natl. Acad. Sci. USA. — 1975. — Vol. 72. — P. 5135—5139.

7. *Singer V.L., Lawlor T.E., Yue S.* Comparison of SYBR Green I nucleic acid gel stain mutagenicity and ethidium bromide mutagenicity in the Salmonella/ mammalian microsome reverse mutation assay (Ames test) // Mut. Res. — 1999. — Vol. 439. — P. 37—47.

8. *Waring M.J.* Complex formation between ethidium bromide and nucleic acids // J. Mol. Biol. — 1965. — Vol. 13. — P. 269—282.

9. *Haughland R.* Molecular Probes. Handbook of Fluorescent Probes and Research Chemicals, Ninth edition, Molecular Probes, Inc, Eugene: OR, 2002.

10. *Yarmoluk S.M., Kovalska V.B., Kryvorotenko D.V., Balanda A.O., Ogul'chansky T.Yu.* Interaction of cyanine dyes with nucleic acids: XXV. Influence of affinity-modifying groups in the structure of benzothiazol-4-[2,6-dimethylpyridinium] dyes on the spectral properties of the dyes in the presence of nucleic acids // Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. — 2001. — Vol. 57. — P. 1533—1540.

11. *Yarmoluk S.M., Kryvorotenko D.V., Kovalska V.B.* Interaction of cyanine dyes with nucleic acids. XX. New methods for preparation of fluorescent probes based on benzothiazol-4-[2,6-dimethylpyridinium] cyanine dyes // Dyes Pigm. — 2001. — Vol. 48. — P. 165—172.

12. *Ficken G., Kendall J.* The influence of bridging of the color of polymethin and aza-methin dyes // Chimia. — 1961. — Vol. 15. — P. 110—115.

13. *Mayboroda E., Slominskiy Yu., Tolmachov A.* Thiocarbocyanines with ethylene and vinylene bridged groups and substituents in chromophore // Ukr. Chim. J. — 2002. — Vol. 68. — P. 106—111.

14. *Ames B.N., McCann J., Yamasaki E.* Methods for detecting carcinigenes and mutagenes with the *Salmonella*/mammalian microsome mutagenicity test // Mutation Res. — 1975. — Vol. 31. — P. 347—364.

15. EC, European Commission. Technical guidance document on risk assessment, European Chemicals Bureau, Office for Official Publications of the European Communities, Luxemburg, 2003.

16. *Schneider K., Hafner C., Jager I.* Mutagenicity of textile dye products // Journal of Applied Toxicology. — 2004. — Vol. 24. — P. 83—91.

17. EPA, United States Environmental Protection Agency Prevention. Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity [August 1998].

18. *Pershin G.N.* Methods of experimental chemotherapy: Practical guidance, Second edition, Medicina, Moscow, 1971.

19. *Chu K.C., Patel K.M., Lin A.H., Tarone R.E., Linhart V.C., Dunkel V.C.* Evaluating statistical analyses and reproducibility of microbial mutagenicity assays // Mutation Res. — 1981. — Vol. 85. — P. 119—132.