MUTAGENIC AND GENOTOXIC ACTIVITY OF CHOSEN DYES AND SURFACE ACTIVE COMPOUNDS USED IN THE TEXTILE INDUSTRY

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Key words: Mutagenicity, Genotoxicity, Micronuclei, Polychromatic erythrocytes, Normochromatic erythrocytes, Azo compounds, Antraquinone compounds

Abstract. This study was designed to investigate the mutagenic and genotoxic properties of ten dyes and four surface active compounds using Salmonella/ /microsome assay and the micronucleus test. Five of the investigated dyes (Acid Blue 7, Acid Green 16, Direct Black 19:1, Basic Red 22, Basic Orange 28) possessed mutagenic activity with regard to test strains of Salmonella. In addition, all of them increased the frequency of micronucleated polychromatic erythrocytes in the bone marrow of mice. Three other compounds (Acid Blue 62, Direct Yellow 12, Direct Red 81), which were not mutagenic in the Salmonella/microsome assay, were genotoxic in the micronucleus test. The other two dyes (Reactive Blue 13, Acid Red 213), as well as tested surface active compounds, did not exert mutagenic and genotoxic effects, and therefore, it is most probable that they do not have carcinogenic properties. Besides, it was noted that Acid Blue 62, Direct Black 19:1, Direct Red 81 and Basic Orange 28 cause a significant decrease in the ratio polychromatic to normochromatic erythrocytes in the bone marrow of mice, which means that, at the doses used in the experiment, they are toxic to the erythrocyte series cells of bone marrow. The other compounds under consideration have no such effect.

INTRODUCTION

It is estimated (17) that about 250 000 new chemical agents are produced yearly and, of these, about 300 are biologically active, and are introduced into our environment without precise knowledge of their mutagenic, genotoxic and carcinogenic activities. If we consider that some similar mechanisms are involved in mutagenesis and carcinogenesis, the early and efficient detection of environmental mutagens would appear to be very important for health protection (3, 9).

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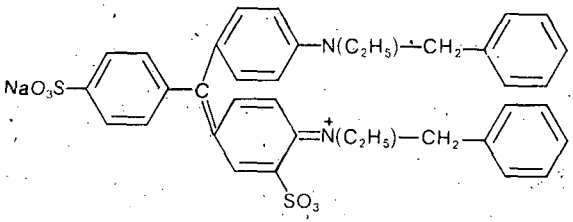
The study was designed to evaluate the mutagenic and genotoxic properties of 10 dyes and 4 surface active compounds using the Salmonella/mammalian microsome assay and the micronucleus test in vivo. The studied dyes are widely used in the textile industry in Poland and a considerable number of workers are exposed to them. Surface active compounds are the additives most frequently used in the textile industry to improve the properties of textile products.

MATERIAL AND METHOD

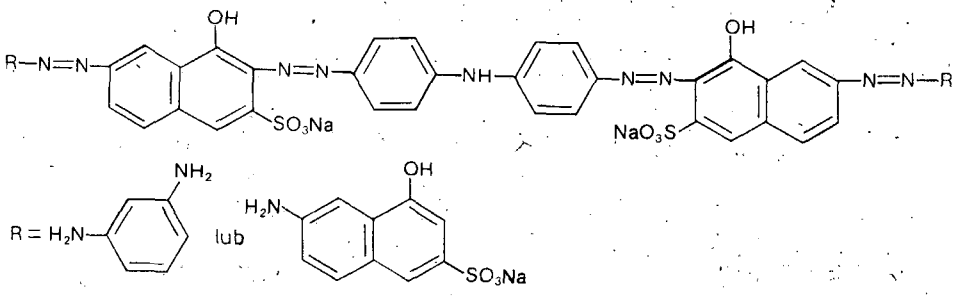
The group of tested compounds included the following dyes — Acid Blue 7 (C.I. 42080), Acid Green 16 (C.I. 44025), Acid Blue 62 (C.I. 62045), Direct Black 19 : 1, Direct Red 81 (C.I. 28160), Direct Yellow 12 (C.I. 24895), Basic Red 22, Basic Orange 28, Reactive Blue 13 (C.I. 61210), Acid Red 213 and surface active compounds — Pretepon G, Rokafenol N-8, Rokacet S-7 and Rokamin S-22. The dyes were tested in their industrial or commercial form. Some of them were also examined in a purified form (by crystallization from ethanol or dimethylformamide solutions) using the Salmonella/microsome assay. The contaminations of technical and commercial preparations consisted of: NaCl, Na_2SO_4, Mn^{2+}, ZN^{2+}, Cu, Na_3PO_4 and Olan UN. The chemical structures of all the examined compounds are shown in Fig. 1.

The Salmonella/microsome test was performed after Ames et al (1) with later modifications (10). Five histidine-dependent strains of S. typhimurium — TA1535, TA100, TA1537, TA1538 and TA98 were used. The tester strains were kindly provided by Professor B. N. Ames (University of California, Berkeley, CA, USA). The sensitivity of the strains was ascertained using appropriate mutagens according to criteria given by Ames et al (1). Mutagens were used as a control in the spot tests: MNNG (1 methyl-3 nitro-1-nitrosoguanidine) for TA1535 strain, 9-aminoacridine for TA1547 strain, NQNO (4-nitroquinoline-N-oxide) for TA100 and 2-nitrofluorene for TA98 and TA1538 without metabolic activation system and 2-aminofluorene with S9-mix fraction.

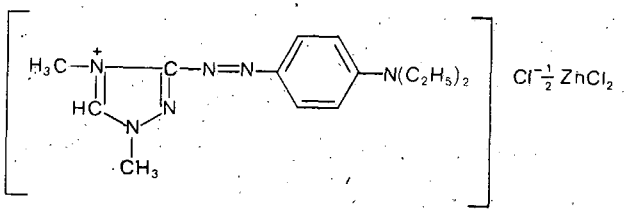
In the Salmonella/microsome assay, all the tested substances were prepared for testing as aqueous or dimethylsulfoxide (DMSO) solutions at the highest concentration which could be prepared. The solutions were diluted where appropriate. All the compounds were tested in two plates per dose in two replicate experiments both in the absence and in the presence of 0.5 cm^3 S9-mix per plate. Three plates were used for spontaneous mutation control assay. S9-mix containing 50 µl S9 in 0.5 cm^3 was prepared from the liver homogenate of rats treated with Aroclor 1254. The amount of proteins, which was determined by the Lowry method (8), was equal to 35.1—46.9 mg per 1 cm^3.



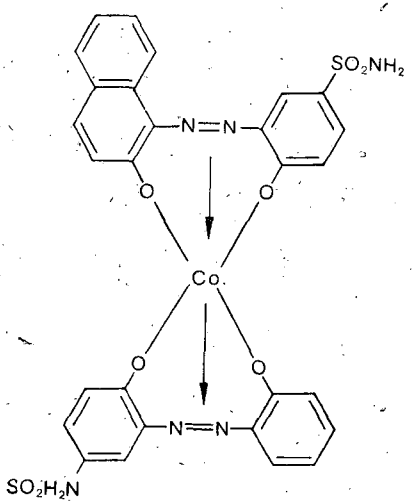
Acid Blue 7



Direct Black 19 : 1

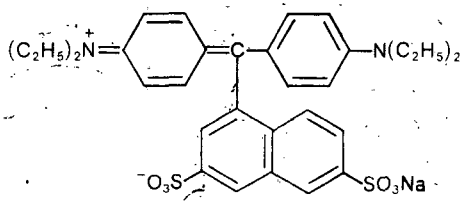


Basic Red 22

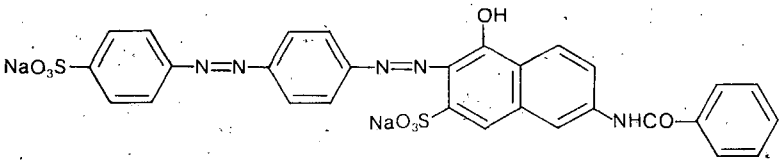


Acid Red 213

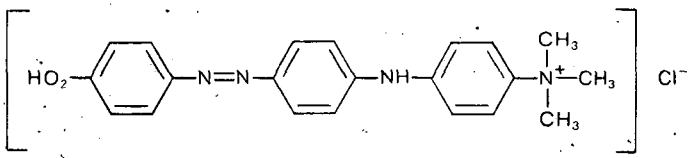
Fig. 1. Chemical structures of the examined compounds.



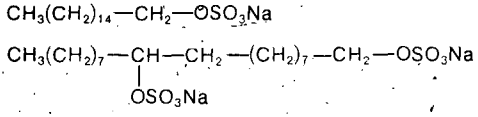
Acid Green 16



Direct Red 81

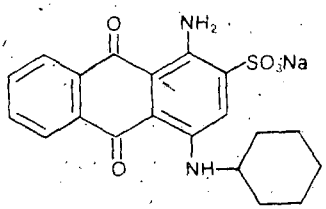


Basic Orange 28

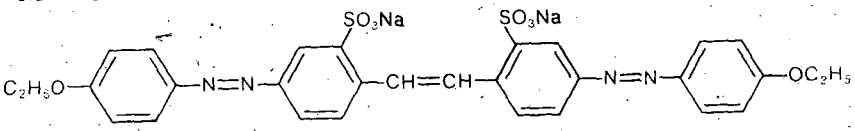


Pretepon G

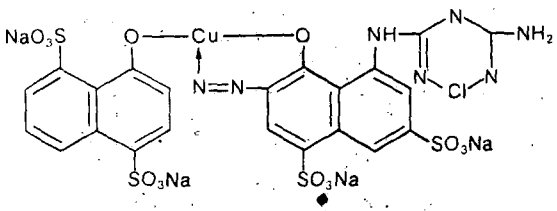
Rokacet S-7



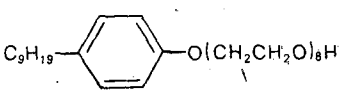
Acid Blue 62



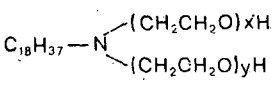
Direct Yellow 12



Reactive Blue 13



Rokafenol N-8



Rokamin S-22

The micronucleus test was carried out on mice of Balb C strain weighing 25—30 g. In preliminary experiments, the LD_{50} (median lethal dose) of all studied compounds administered i.p. to male mice was determined using the Thompson and Weil method (19). In the micronucleus test, the examined substances were dissolved in 0.9% aqueous solution of NaCl or in DMSO just before treatment, and administered to male mice of the Balb C strain weighing 25—30 g by i.p. injections, each dose administered in two equal portions separated by 24 h interval. The negative control group of animals received only 0.9% aqueous solution of NaCl or DMSO used as a solvent. Mitomycin C (Sigma, London, U.K.) dissolved in 0.9% NaCl was administered to the animals in the positive control group. Each group of animals consisted of four male mice. Some dyes (Acid Blue 7, Acid Green 16, Acid Blue 62, Direct Black 19 : 1, Direct Red 81, Basic Red 22) were tested in dose equal to approximately 80% LD_{50} and 4—5 appropriate lower doses according to the procedure described by Schmid (13). Bone marrow was sampled 30 h after the first injection. The other compounds were tested in a two-phase experimental model as recommended by Heddle et al (7). In the first phase, the chemical was given to male mice in doses equal to about 80% LD_{50}. Bone marrow was sampled at 30,48 and 72 hours after the first injection. In the second phase, the positive results were confirmed by a dose-response study at the time of the maximum response, but the negative results were established using the same treatment schedule as in the first phase of the experiment.

The preparation of bone marrow and staining were carried out after Schmid (13) with slight modifications: 1% aqueous solution of sodium citrate was used instead of foetal calf serum and slides were stained in Giemsa (Merck) diluted 1:10 in Sörensen buffer (pH=6.8).

The number of micronucleated cells in at least 1000 polychromatic erythrocytes, as well as the ratio of normochromatic to polychromatic erythrocytes, were determined for each animal.

In statistical analysis of the results, the Wilcoxon test or one-way analysis of variance, were used (6, 16).

The compound was considered to be positive when the dose-response relationship and/or a statistically significant and reproducible increase in the number of micronucleated polychromatic erythrocytes were observed for at least one of the points.

RESULTS

The positive results of the Salmonella/microsome assay are presented in Tables 1 and 2. Among the dyes examined in this test, five — Acid Blue 7, Acid Green 16, Direct Black 19:1, Basic Red 22 and Basic Oran-

ge 28 induced a dose-related increase in the number of revertants. Direct Black 19:1 and Basic Orange 28 in both industrial and purified forms and Basic Red 22 in purified form acted as direct mutagens inducing frameshift reverse mutations. Additionally, Basic Orange 28 in technical form was responsible for the induction of base pair mutations in the S. typhimurium TA100, but only at very high dosages. Acid Blue 7, Acid Green 16 in their technical and purified forms and Basic Red 22 in its technical form acted as an indirect mutagen, inducing frameshift mutations in strains TA1538 and TA98. Acid Blue 7, Acid Green 16, Direct Black 19:1, and Basic Red 22, but not Acid Green 16 and Basic Orange 28, were toxic to used bacteria at higher doses. The rest of the studied dyes (Acid Blue 62, Direct Red 81, Direct Yellow 12, Reactive Blue 13, Acid Red 213), as well as the other investigated compounds (Pretepon G, Rokafenol N-8, Rokacet S-7 and Rokamin S-22), did not induce his^+ reverse mutations in any of the strains of S. typhimurium employed.

All the compounds mutagenic in the Salmonella/microsome assay (Acid Blue 7, Acid Green 16, Basic Red 22, Direct Black 19:1, Basic Orange 28) and three other dyes not mutagenic in bacteria (Acid Blue 62, Direct Yellow 12, Direct Red 81), caused a significant and dose-related increase in the frequency of polychromatic erythrocytes with micronuclei in the bone marrow of mice (Table 3). Reactive Blue 13 and Acid Red 213 and surface active compounds (Pretepon G, Rokafenol N-8, Rokaced S-7 and Rokamin S-22) did not induce any increase in the percentage of polychromatic erythrocytes with micronuclei.

The experiment with Mitomycin C confirmed the sensitivity of the applied experimental model for detecting chemical mutagens, since Mitomycin C at doses 0.65—5.20 mg/kg body weight demonstrated a significant dose-related increase in the percentage of micronucleated polychromatic erythrocytes, as compared to the negative control value (Table 3).

The decrease in the ratio of the polychromatic to normochromatic erythrocytes which was induced by Acid Blue 62, Direct Black 19:1, Direct Red 81, Direct Yellow 12, and Basic Orange 28 indicated the toxicity of these compounds to the erythrocyte series cells of bone marrow (Table 3).

The median lethal dose of the genotoxic substances for male mice are shown in Table 3. One of examined surface-active compounds, Rokacet S-7, did not kill any mice at a dose equal to 14000 mg/kg.

LD_{50} of the rest of studied dyes — Reactive Blue 13, Acid Red 213 and surface-active additives, Pretepon G, Rokafenol N-8, Rokamin S-22, amounted to 144, 1060 and 1750, 249,177 mg/kg, respectively.

TABLE 1. The Results of Mutagenicity Study of Technical or Commercial Dyes in Salmonella/Microsome Assay

Compound	Dose level µg/plate	His+ colonies/plate									
		TA1535		TA100		TA1537		TA1538		TA98	
		−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Acid Blue 7	0	16±2	14±3	81±5	106±4	6±3	7±2	14±2	23±16	16±4	27±4
	100	21±4	16±8	82±8	96±5	9±2	8±4	15±5	**46±9**	16±6	43±5
	250	19±3	12±1	NT	NT	6±1	7±1	14±1	**98±16**	16±1	**66±6**
	500	22±0	6±1	84±17	101±7	5±1	7±2	17±1	**149±17**	16±3	**105±15**
	1000	NT	NT	84±3	85±7	NT	NT	13±4	**157±1**	15±2	**101±2**
	2500	15±4	8±1	NT	NT	4±1	4±0	13±1	11±3	12±1	14±1
Acid Green 16	0	16±3	11±3	116±31	102±20	7±2	14±5	19±7	31±7	15±3	38±6
	500	23±1	14±3	109±14	83±8	10±4	8±1	20±2	**113±41**	17±3	**76±17**
	1000	21±1	9±3	106±11	89±3	7±5	14±3	18±5	**176±60**	14±6	**114±39**
	2500	16±9	12±3	93±17	92±8	6±1	15±6	13±4	**310±104**	19±7	**166±28**
	5000	13±6	10±6	95±11	101±3	7±1	21±1	19±3	**217±44**	16±4	**148±49**

Direct Black 19:1	0	25±3	11±5	106±8	87±10	6±3	9±2	18±3	28±5	29±7	37±9
	5	27±0	11±3	104±12	76±13	8±7	10±4	21±6	60±9	29±11	57±5
	50	28±1	12±3	123±55	98±6	9±1	16±3	23±7	169±31	32±8	103±36
	100	33±8	10±1	94±11	114±13	9±0	16±4	23±5	302±58	35±15	114±58
	500	15±0	9±2	81±1	91±6	6±2	12±3	26±2	133±85	41±7	62±49
	1000	NT	NT	NT	NT	NT	NT	32±7	25±3	28±9	26±6
	2500	16±2	7±1	88±2	95±13	9±4	7±3	58±9	36±14	47±22	29±6
Basic Red 22	0	18±2	11±3	105±13	93±4	8±3	9±3	18±5	28±5	20±6	32±7
	50	14±6	13±0	106±4	117±6	10±3	11±3	25±10	79±13	22±8	79±24
	250	16±4	15±3	113±20	118±3	8±1	31±7	23±10	216±52	24±6	218±105
	500	20±0	11±3	112±12	158±13	9±3	51±26	32±4	279±67	26±8	286±94
	2500	11±5	14±1	121±7	104±2	11±3	15±5	30±15	87±18	35±10	52±9
Basic Orange 28	0	18±4	14±4	140±9	144±17	7±2	7±3	15±3	22±3	19±5	33±9
	10	19±6	11±2	135±14	135±15	7±3	7±4	47±9	48±4	30±5	36±10
	100	17±2	16±2	153±6	174±5	22±7	16±3	412±57	404±72	167±56	141±31
	250	20±1	14±4	178±2	194±13	27±3	24±6	668±179	921±109	439±91	469±161
	500	15±5	19±1	205±38	254±28	35±19	50±17	768±148	1306±39	848±60	1065±302
	1000	14±1	11±1	235±51	280±44	38±14	55±26	1174±138	1648±519	1516±275	1937±272

Numbers underlined indicate mutagenic effect; −S9 and +S9 indicate absence or presence of metabolic activation system. Values presented are means from at least four plates in two separate experiments except negative control where six plates were used.

TABLE 2. The Results of Mutagenicity Study of Purified Dyes in Salmonella/Microsome Assay

Compound	Dose level µg/plate	His+ colonies/plate									
		TA1535		TA100		TA1537		TA1538		TA98	
		−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Acid Blue 7	0	15±5	13±2	97±4	109±18	6±3	7±2	16±4	25±16	19±2	32±7
	100	20±10	12±3	104±1	108±3	9±1	5±2	18±5	**55±10**	17±2	43±17
	250	25±6	6±3	92±5	107±7	9±7	8±5	17±2	**92±11**	22±3	**74±10**
	500	25±1	11±3	108±3	94±4	7±3	7±0	14±4	**183±9**	12±3	**120±24**
	1000	NT	NT	NT	NT	NT	NT	15±6	**183±16**	9±1	**203±26**
	2500	21±4	10±3	109±11	84±15	6±3	6±5	14±3	28±2	19±6	18±3
Acid Green 16	0	16±6	14±2	112±2	110±9	10±4	11±2	28±9	31±7	26±3	38±6
	500	13±1	12±5	106±16	113±2	9±4	6±1	27±2	**135±52**	21±2	**78±14**
	1000	17±4	7±5	123±19	141±15	7±4	13±1	18±6	**223±59**	21±7	**134±52**
	2500	18±1	4±1	99±10	136±20	6±1	10±6	19±2	**274±49**	19±11	**204±75**
	5000	16±1	5±1	92±3	112±15	5±3	16±7	26±2	**127±46**	15±3	**126±25**

Direct Black 19 : 1	0	25±3	11±5	106±8	87±10	6±3	9±2	18±3	28±5	29±7	37±9
	5	27±0	11±3	104±12	76±13	8±7	10±4	21±6	**60±9**	29±11	57±5
	50	28±1	12±3	123±55	98±6	9±1	16±3	23±7	**169±31**	32±8	**103±58**
	100	33±8	10±1	94±11	114±13	9±0	16±4	23±5	**302±58**	35±15	**114±58**
	500	15±0	9±2	81±1	91±6	6±2	12±3	26±2	**133±85**	41±7	62±49
	1000	NT	NT	NT	NT	NT	NT	32±7	25±3	28±9	26±6
	2500	16±2	7±1	88±2	95±13	9±4	7±3	**58±9**	36±14	47±22	29±6
Basic Red 22	0	22±5	13±3	105±9	114±7	7±3	9±3	20±5	26±4	21±6	33±6
	50	25±5	15±2	103±36	139±16	8±2	**26±1**	30±9	**216±137**	31±8	**243±50**
	250	29±6	13±4	114±15	126±5	9±2	17±3	**79±36**	**366±43**	**47±14**	**243±63**
	500	21±8	9±5	114±13	132±17	13±5	**25±4**	**149±57**	**325±43**	**66±25**	**179±41**
	2500	16±4	11±2	101±7	141±8	**25±14**	**33±11**	**92±29**	**461±143**	173±83	**229±90**
Basic Orange 28	0	17±4	14±5	145±12	148±5	7±2	6±2	15±3	22±3	19±5	33±9
	10	16±4	15±4	136±7	137±21	8±2	**12±5**	**37±11**	38±17	34±6	35±4
	100	18±3	17±6	154±7	158±8	**15±10**	**14±3**	**148±9**	**123±51**	**80±14**	**83±19**
	250	17±7	17±4	156±10	164±7	**16±4**	**13±4**	**191±13**	**230±68**	**144±50**	**152±38**
	500	18±7	11±3	155±23	176±1	**12±2**	**19±1**	**333±86**	**333±31**	**263±7**	**237±8**
	1000	17±3	14±2	183±27	173±27	**16±6**	**17±4**	**355±75**	**611±52**	**442±103**	**457±6**

Numbers underline indicate mutagenic effect; −S9 and +S9 indicate absence or presence of metabolic activation system. Values presented are means from at least four plates in two separate experiments except negative control where six plates were used.

TABLE 3. The Frequency of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Balb C Mice and the Ratio of Normochromatic to Polychromatic Erythrocytes

Compound	LD_{50} (mg/kg)	Total dose (mg/kg)	Number of polychromatic erythrocytes with micronuclei (% ±SD)	Ratio of normachromatic to polychromatic erythrocytes
Acid Blue 7 (C.I. 42080)	437	0	0.40±0.15	
		37.5	1.07±0.38 *	
		75	1.52±0.49 *	
		150	2.01±0.59 *	n.e.
		300	2.10±0.19 *	
		600	2.59±0.55 *	
Acid Green 16 (C.I. 44025)	892	0	0.41±0.12	
		75	1.42±0.50 *	
		150	1.72±0.33 *	
		300	2.04±0.45 *	n.e.
		600	2.72±0.79 *	
		1200	2.93±0.11 *	
Acid Blue 62 (C.I. 62045)	783	0	0.42±0.12	1.03
		87.5	0.63±0.02 *	0.98
		175	0.73±0.25 *	0.93
		350	1.10±0.26 *	1.26
		700	2.09±0.18 *	1.60 *
		1400	2.40±0.16 *	1.64 *
Direct Black 19:1	1000	0	0.42±0.12	1.03
		16.6	0.80±0.11 *	1.02
		50	0.82±0.37 *	1.48
		150	0.94±0.11 *	1.22
		450	0.36±0.23 *	1.45 *
		1350	1.54±0.39 *	1.37 *
Direct Red 81 (C.I. 28160)	1048	0	0.42±0.12	1.03
		37	0.99±0.18 *	1.15
		111	1.58±0.79 *	1.12
		333	2.10±0.24 *	1.28
		1000	2.58±0.83 *	1.81 *
		3000	2.22±1.41 *	1.74 *
Direct Yellow 12	320	0	0.65±0.05	0.97
		20	1.17±0.40	1.29
		40	1.75±0.41 *	1.15
		80	1.90±0.52 *	1.33 *
		160	2.50±0.27 *	2.17 *
		320	3.40±0.84 *	2.61

Basic Red 22	99	0	0.41±0.12	n.e.
		8.75	0.91±0.48	
		17.50	1.38±0.54 *	
		35	1.92±0.85 *	
		70	1.87±0.44 *	
		140	1.93±0.92 *	
Basic Orange 28	280	0	0.65±0.05	0.97
		17.5	0.97±0.49	0.93
		35	1.42±0.22 *	0.94
		70	2.02±0.62 *	1.44 *
		140	2.30±0.16 *	1.57 *
		280	1.60±0.29 *	1.66 *
Mitomycin C (Sigma, London)		0	0.65±0.05	0.97
		2.50	2.85±0.28 *	1.60 *
		5.00	3.90±0.32 *	1.80 *

* — Difference statistically significant ($p < 0.05$)
n.e. — not examined

DISCUSSION AND CONCLUSIONS

The results of the median lethal dose study indicate that the toxicity of the investigated dyes is rather low and that none of them seems to cause a risk of acute occupational poisoning for man. However, some of these dyes possess mutagenic and/or genotoxic activity and besides they are responsible for a toxic effect on the erythrocyte series cells of bone marrow at a dose close to their LD_{50}. The toxic effect on these cells was observed only after doses higher than genotoxic ones had been used.

Of the six examined azo compounds (Direct Black 19:1, Direct Red 81, Direct Yellow 12, Basic Red 22, Basic Orange 28, Acid Red 213), the first five possess mutagenic and/or genotoxic activity. The results obtained from other studies (4, 5) indicate that three tetraazo dyes: Direct Blue 6, Direct Black 38 and Direct Brown 95 — which are structurally related to azo dyes — found to be genotoxic in our study, were active as rat liver carcinogens. In addition, some other azo compounds are reported to show carcinogenic activity (11, 12). Therefore, it is very likely that all mutagenic and/or genotoxic azo dyes are also carcinogenic.

Carcinogenic properties may also be possessed by two triaryl compounds under examination: Acid Blue 7 and Acid Green 16, which induced his reverse mutation in tested strains of S. typhimurium and produced the increase of micronucleated erythrocytes in bone marrow of mice.

One of the antraquinone dyes tested: Acid Blue 62, which was not mutagenic in S. typhimurium tester strains in this and Venturi and

Tamaro studies (18), increased the frequency of micronucleated erythrocytes. Antraquinone derivatives are structurally related to anthracycline antitumor antibiotics of known mutagenicity and carcinogenicity (2, 14, 15). Therefore, the genotoxic activity of Acid Blue 62 in the micronucleus test may indicate the derivatives' capability of damaging genetic material in mammals. However, taking into account the possibility of false positive results, the substances inducing genetic effect in only one experimental model require further testing for sufficient assessment of their genotoxicity.

The substances with proven genetic toxicity to bacterial and mammalian cells may also damage human genetic material. Thus, the occupational exposure to such compounds in the workplace ought to be limited as far as possible. Reactive Blue 13, Acid Red 213, Pretepon G, Rokafenol N-8, Rokacet S-7 and Rokamin S-22 which did not exert mutagenic and genotoxic effects most probably do not threaten genetic damage in man. The evidence obtained on the genetic toxicity of the substances tested in this study does not prove their carcinogenic properties, which should be assessed in long-term tests on animals.

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