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GENOTOXICITY ASSESSMENT OF SULFOSUCCINATE IO-5, ROKAMID MRZ 17, ROKACET RZG7P2 AND ROKSOL TL-7 USING BACTERIA AND BONE MARROW RODENT CELLS

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Abstract. Three short-term tests were used for evaluation of the genotoxic activity of four surface active agents. These were: Ames, Salmonella reversion assay using 4 tester strains (TA97a, TA98, TA100 and TA102), the micronucleus test int the bone marrow of Balb C mice and *in vivo* sister chromatid exchange (SCE) analysis in SFIS or Balb C mice. The frequency of micronucleated PCEs did not exceed the control values in mice of both sexes after intraperitoneal administration of all four compounds. Three preparations – Sulfosuccinate IO-5, Rokamid MRZ 17 and Rokacet RZG7P2 produced a negative response in Salmonella strains gene mutation assay and SCE induction test in mouse bone marrow cells. The Roksol TL-7 induced frameshift and base-pair substitution mutations in Salmonella tester strains both with and without S9 (prepared from the liver of rats which had been pretreated with Aroclor 1254). Evidently positive results (more than a twofold increase in the number of revertants per plate) were observed in tester strain S. typhimurium TA97a (with and without S9 metabolic activation) and S. typhimurium TA100 (with S9 metabolic activation)at a dose of 0,2 µl Roksol TL-7 per plate.Roksol TL-7 caused slight increase in the SCE level in mouse bone marrow cells. A significant increase in SCE frequency was observed at doses of 50, 75 and 100 mg/kg

INTRODUCTION

This study was aimed at determining the genotoxic activity of four surface active agents: Sulfosuccinate IO-5, Rokamid MRZ 17, Rokacet RZG7P2 and Roksol TL-7.

These preparations are widely used in household chemistry and cosmetic products. Sulfosuccinate IO-5, among others, is a componet of bath lotions.

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Rokamid MRZ 17, as a noionic surface-active agent, is added to detergents or it serves as emulgator in various oiling compositions. Roksol TL-7 serves as the corrosion inhibitor in industrial liquids, especially in water-oil emulsions.

To evaluate the biological activity of these four compounds three test assays were used: i.e. Ames, Salmonella reversion assay for the effects on gene mutations, the bone marrow micronucleus assay in mice for the effects on chromosome breaks and spindle dysfunctions and the sister chromatid exchange (SCE) induction test for the effects on the SCE frequency in mouse bone marrow cells.

Chemicals

The test surface active agents used in the study were provided by the Organika-Rokita Chemical Plant.

Four preparations were studied: Sulfosuccinate IO-5 (aqueous solution of sulfosuccinate and oxyalkylated isooctylic alcohol), Rokamid MRZ 17 (ethoxylated ethanolamides acids of colza oil), Rokacet RZG7P2 (oxyalkylated glycerides of fatty acids) and Roksol TL-7 with the essential ingredient 1,3,3 tri-(2-hydroxyethyl) hexahydrotriazine. All of the preparations tested are liquids. Freshly prepared solutions (the preparations were diluted in distilled water or 0,9% NaCl) were used for each experiment.

Toxicity test

The concentrations of surface active agents used in the mutagenicity study were based on preliminary experiments to determine the limit of solubility and toxicity. The preparations exhibiting toxic effect (recorded as inhibition of the background lawn of bacteria and as a decrease, below 75%, in the number of revertants as compared to the spontaneous number) were limited in the dose range of testing. In cases of high toxicity, to prove that the colonies counted were true revertans, we transferred the individual colonies onto minimal agar plates without histidine to demonstrate their His^+ genotype.

A range of doses in the micronucleus and SCE test was determined by a preliminary study to assess the toxicity of compounds (LD_{50}) according to the method of Thompson-Weil (9) using the intraperitoneal exposure route.

LD_{50} for the test preparations were: Sulfosuccinate IO-5 for male mice 2510 mg/kg and female mice 1435 mg/kg; Rokamid MRZ 17 for males 715 mg/kg and for females 635 mg/kg; Roksol TL-7 for males and females the same dose level 320 mh/kg and 125 mg/kg found for male mice of Balb C strain (the study was made in other time); Rokacet RZG7P2 at the highest dose 14000 mg/kg did not produce death of the mice.

Salmonella reversion assay

The plate incorporation assay for mutation reversion in four S. typhimurium strains (TA97a, TA98, TA100 and TA102) to histidine independence was performed essentially according to the method of Ames et al. (2) as revised by Maron and Ames (7). The strains were obtained from the laboratory of Prof. Bruce N. Ames (Berkeley,

California, USA). Tests were carried out in the absence and presence of S9-mix (50µl S9 per plate with the necessary standard cofactors) obtained from the livers of Aroclor 1254-pretreated male Wistar rats Imp: DAK. The protein content in S9 fraction used in these investigations was between 35–39 mg/ml as determined by the Lowry method with bovine serum albumins as the standard (5).

The bacteria were grown in "Oxoid" medium for 10h only. In each single test, 2 plates per dose-point were used for the compounds and the positive controls and 3 plates for the solvent and spontaneous controls. To confirm the sensitivity of the test system to known mutagens, the following positive controls were concurrently tested: 4-Nitroquinoline-N-oxide at 05µg/plate (TA97a, TA102), 4-Nitro-o-phenyloendiamine at 3 µg/plate (TA98), Sodium azide at 1,5 µg/plate (TA100) without S9 metabolic activition and 2-Aminofluorene at 5 µg/plate for all strains with S9 metabolic activation. Each test was duplicated in independent experiments to ensure reproducibility. The plates were incubated for 2 days at 37°C and colonies of His^+ revertants were counted with an automated colony counter Biotran II (New Brunswick Scientific Co., NJ). The test preparations were dissolved in water except for Rokacet RZG7P2 which was dissolved in 95% ethanol. The doubling of the spontaneous revertants rate was a criterion to confirm the mutagenic effect.

The micronucleus test

All experiments were performed on male and femele Balb C mice of body weight about 25–30 g. The test preparations were dissolved in 0.9% Na Cl and were administered i.p. in a volume of 0.5 ml/animal. Doses of 80% and 40% of the respecitive LD_{50} dose (Sulfosuccinate IO-5, Rokamid MRZ 17 and Roksol TL-7) were used in the micronucleus test as described in detail by Przybojewska (8). Rokacet RZG7P2 was tested at the highest dose 14 000 mg/kg and at a dose level equal to 50% of it (7000 mg/kg). Five mice (4 mice for a positive control) were employed for each dose of preparation at each harvest time (30, 48 and 72 h after the first administration). Each dose of test preparation was divided and administered to the animals in two identical parts at 24h intervals. Mice from the control groups were given: 1 ml 0.9% NaCl or Methyl methanesulfonate (150 mg/kg). Thousand bone marrow polichromatic erythrocytes (PCEs) per animal were analysed for incidence of polychromatic erythrocytes with micronuclei; 500 cells from each mouse were counted to determine the ratio of polychromatic to normochromatic erythrocytes (PCEs/NCEs). The statistical analysis of the results was performed by the one-way analysis of variance with the multiple comparison test (4).

A statistically significant and reproducible increase in the number of micronucleated polychromatic erythrocytes observed for, at least, one of the points was a criterion confirming the genotoxic effect.

The SCE test

For the SCE frequency analysis, male SFIS or Balb C mice, 11–12 week old, weighing about 25–30 g were used. The assay method was used according to the procedures: Allen et al. (1) and Latt et al. (6). SCE frequencies were analyzed in mouse bone marrow cells after DNA labelling with 5-bromodeoxyuridine (BrdU).

Into anesthetized mice, tablets of 50 mg BrdU (Serva) were implanted subcutaneously. An hour later, test preparations were given as a single (0.5 ml volume) i.p. injection to 5 mice (4 mice for a positive control) per dose. Four doses of about 80%, 60%, 40% and 20% of the respective LD_{50} dose (Sulfosuccinate IO-5, Rokamid MRZ 17 and Roksol TL-7) or of the highest nonlethal dose 14 000 m/kg of Rokacet RZG7P2 were used. As a negative control, the mice were treated with H_2O or 0.9% NaCl (solvent control). Positive control mice were treated with 10 and 15 mg/kg Cyclophosphamide. Twenty hours after administration of tested preparations and two hours prior to the killing of the animals, Colcemid was injected i.p. at the amount of 3.2 mg/kg b.w. The animals were killed by cervical dislocation, bone marrow cells were harvested and SCE slides prepared according to Antoshina and Porjadkova (3). For evaluation of the effects *in vivo* of the test preparations on SCE ferquency 50 metaphase cells were analysed.

To compare the SCE frequency per cell in the treatment groups with that of the negative control, the statistical estimation of one-way analysis of variance with a multiple comparison test (4) was employed. Differences were considered statistically significant when $p<0.05$.

RESULTS

The results of the mutagenicity test of four preparations in Salmonella typhimurium TA97a. TA98, TA100 and TA102 including negative and positive controls are given in Table 1. The results show that three surface active agents (Sulfosuccinate IO-5, Rokamid MRZ17 and Rokacet RZG7P2) did not produce positive (that is more than twofold) increases in the numbers of revertant colonies over the spontaneous rate with and without S9 metabolic activation.

As compared to the control value, the revertant numbers were slightly increased at higher doses of Roksol TL-7 in all of the tester strains. At doses higher than 0.2 and 0.3 µl per plate the revertant numbers decreased due to toxicity to the bacteria as indicated. The response obtained with bacterial strains was dose-related but relatively weak. Although a positive trend was observed with Roksol TL-7 concentrates, the data in Table 1 show that preparation at a dose of 0.2 µl per plate was able to induce more than a twofold increase in the spontaneous number of revertants, an approx, threefold overbackground in TA97a (with and without S9) and an approx, 3.5-fold overbackground in TA100 (with S9).

The results of the micronucleus test are summarised in Table 2. In Balb C mice of both sexes treated with various doses of the test preparations the frequency of micronucleated PCEs did not increase over the control value. A highly significant ($p<0.05$) increase was observed in male and female positive control groups treated with 150 mg/kg Methyl methanesulfonate. All of the test preparations were not toxic to bone marrow as shown. The PCEs/NCEs ratio (an index measuring bone marrow citotoxicity resulting from exposure to the test compounds) was not considerably higher than that found in control mice injected with 0.9% NaCl.

The results of analyses of mouse bone marrow cell SCE frequencies are presented in Table 3. As expected an increase in sister chromatid exchanges per cell was observed in cells exposed to 10 and 15 mg/kg Cyclophosphamide. There was no

Table 1. Average histidine revertants per plate for Salmonella typhimurium strains.

Preparation	Dose per plate μl or μg	Number of revertants per plate with standard deviation							
		TA97a		TA98		TA100		TA102	
		−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
	0	133±27	134±13	20±2	31± 5	123±12	138±18	292±24	384±54
Sulfosuccinate	0.1	120± 4	136± 5	25±3	32± 8	139±14	131±18	335±41	420±36
IO-5	1.0	111±10	134±18	22±4	35± 4	120±10	128±15	368±24	428±10
	5.0	122±13	141± 8	25±7	33± 6	136±23	154±13	367±32	431±23
	10.0	116± 9	126±16	20±4	38± 6	133±10	136± 6	310±23	385±10
	0	133±27	134±13	20±2	31± 5	123±12	138±18	274±34	351±25
	0.005	NT	NT	NT	NT	NT	NT	288±48	336±30
Rokamid	0.01	NT	NT	NT	NT	NT	NT	279±42	340±16
MRZ 17	0.1	120±16	132±19	25±1	34± 5	134± 5	150±14	217±31	362±31
	0.5	NT	NT	NT	NT	NT	NT	109± 8	234± 9
	1.0	120± 9	118±20	22±4	32± 6	130± 6	134±16	Tox	113±21
	5.0	121± 5	108± 9	21±2	26±10	123±16	126±19		
	10.0	95± 6	84±18	16±4	22± 1	132±15	136±15		
	0	123±17	132± 8	22±3	34± 3	119±11	122±10	301±27	353±20
Solvent control (95% ethanol)	100.0	113±21	137±22	22±3	37± 2	119±13	132±15	298±19	348±24
Rokacet	0.1	118±23	150±20	21±1	37± 4	105±13	133±27	314±14	403±17
RZG7P2	1.0	122±20	131±10	26±2	32± 4	114±13	137± 4	322± 6	393±20
	5.0	120±17	157± 7	24±7	32± 1	104± 4	132±10	329± 9	413±37
	50.0	121±13	136± 6	19±2	44± 5	110±15	134± 6	352±16	403±20
	0	122±18	135±13	26±6	36± 6	124±15	129±12	292±26	378±45
Roksol	0.01	114± 9	127±13	24±3	36± 5	124±23	139±12	373±28	445±33
TL-7	0.05	144±27	151±20	24±4	39±10	139±14	141±11	466±54	498±66
	0.1	197±44	166±14	37±7	42± 7	235±31	250±47	418±63	593±41
	0.2	355±33	391±45	Tox	44±10	240±27	436±43	503±73	712±73
	0.3	Tox	208±72	Tox	Tox	Tox	Tox	Tox	582±37
Positive controls:									
4-Nitroquinoline--N-oxide	0.5	513±71						592±20	
4-Nitro-o-phenylenediamine	3.0			308±26					
Sodium azide	1.5					915±64			
2-Aminonofluorene	5.0	138±18	626±57	26±5	1099±67	130±14	780±82	278±32	640±47

NT — not tested,
Tox — toxic at tested concentration.
+S9 — indicates the presence of S9-mix containing 50 μl Aroclor 1254-induced rats liver S9 per plate.

Table 2. The induction of micronuclei in polychromatic erythrocytes of bone marrow of Balb C mice.

Test preparation	Treatment mg/kg	Number of mice (sex)	Harvest time (h)	% mPCEs ± SD		Ratio PCEs/NCEs	
				male	female	male	female
0.9% NaCl	1 ml	5(M) 5(F)	48	0.18 ± 0.12	0.14 ± 0.06	1.43	1.43
Sulfosuccinate	1005	5(M)	30	0.14 ± 0.04		1.43	
IO-5		5(M)	48	0.18 ± 0.08		1.48	
		5(M)	72	0.10 ± 0.10		1.34	
	1150	5(F)	30		0.14 ± 0.06		1.62
		5(F)	48		0.10 ± 0.10		1.39
		5(F)	72		0.16 ± 0.04		1.50
	2010	10(M)	30	0.10 ± 0.10		1.49	
		10(M)	48	0.13 ± 0.07		1.55	
		10(M)	72	0.14 ± 0.07		1.32	
0.9% NaCl	1 ml	5(M) 5(F)	48	0.12 ± 0.08	0.14 ± 0.06	1.37	1.43
Rokamid	285	5(M)	30	0.16 ± 0.06		1.69	
MRZ 17		5(M)	48	0.12 ± 0.08		1.35	
		5(M)	72	0.14 ± 0.06		1.67	
	510	5(F)	30		0.14 ± 0.06		1.60
		5(F)	48		0.10 ± 0.10		1.56
		5(F)	72		0.14 ± 0.06		1.81
	570	10(M)	30	0.17 ± 0.07		1.61	
		10(M)	48	0.13 ± 0.06		1.25	
		10(M)	72	0.15 ± 0.04		1.56	
0.9% NaCl	1 ml	5(M) 5(F)	48	0.16 ± 0.05	0.15 ± 0.06	1.29	1.08
Rokacet	7000	5(M)	30	0.14 ± 0.07		1.35	
RZG7P2		5(M)	48	0.12 ± 0.06		1.08	
		5(M)	72	0.10 ± 0.10		1.24	
	14 000	10(M) 5(F)	30	0.14 ± 0.07	0.12 ± 0.10	1.36	0.98
		10(M) 5(F)	48	0.16 ± 0.08	0.14 ± 0.07	1.24	1.04
		10(M) 5(F)	72	0.12 ± 0.06	0.08 ± 0.06	1.19	1.03
9.0% NaCl	1 ml	5(M) 5(F)	48	0.14 ± 0.05	0.12 ± 0.10	1.31	1.06
Roksol TL-7	130	5(M)	30	0.18 ± 0.06		1.32	
		5(M)	48	0.18 ± 0.06		1.58	
		5(M)	72	0.14 ± 0.08		1.44	
	255	10(M) 5(F)	30	0.14 ± 0.07	0.14 ± 0.09	1.32	1.24
		10(M) 5(F)	48	0.15 ± 0.10	0.10 ± 0.10	1.48	1.35
		10(M) 5(F)	72	0.16 ± 0.08	0.10 ± 0.04	1.43	1.34
Solvent control							
(0.9% NaCl)	1 ml	5(M)	30	0.12 ± 0.08		1.51	
		5(F)	48		0.12 ± 0.10		1.06
Positive control		4(M) 4(F)	30	2.44 ± 0.56*	2.45 ± 0.66*	1.30	1.05
(Methyl methane-	150	4(F)	48		2.35 ± 0.25*		1.06
sulfonate)		4(F)	72		0.23 ± 0.17		0.83

* — signifaicant difference vs. control at $p < 0.05$.

Table 3. SCE frequency in mouse bone marrow cells.

Test preparation	Treatment mg/kg	Number of mice (strain)	SCE/cell mean ±SD (number of cells)
Solvent control (H_2O)	0.5 ml	5 (SFIS)	4.32±2.09 (250)
Sulfosuccinate	500	5 (SFIS)	4.39±1.97 (250)
IO-5	1005	5 (SFIS)	5.22±2,57 (250)
	1510	5 (SFIS)	5.51±2.18 (230)
	2010	5 (SFIS)	All mouse died
Solvent control (H_2O)	0.5 ml	5 (SFIS)	4.32±2.09 (250)
Rokamid MRZ 17	145	5 (SFIS)	5.15±2.59 (250)
	290	5 (SFIS)	4.92±2.30 (250)
	430	5 (SFIS)	4.67±2.10 (250)
	570	5 (SFIS)	4.78±2.43 (250)
Solvent control (0.9% NaCl)	0.5 ml	5 (Balb C)	4.09±1.84 (250)
Rokacet RZG7P2	2800	5 (Balb C)	4.11±1.80 (250)
	5600	5 (Balb C)	4.26±1.87 (250)
	8400	5 (Balb C)	4.54±1.94 (250)
	11 200	5 (Balb C)	4.60±2.03 (250)
Solvent control (H_2O)	0.5 ml	5 (Balb C)	4.11±1.96 (250)
Roksol TL-7	25	5 (Balb C)	4.84±1.87 (250)
	50	5 (Balb C)	5.42±2.25[c] (250)
	75	5 (Balb C)	6.12±2.64[c] (250)
	100	5 (Balb C)	6.70±2.42[c] (250)
Positive control	10	4 (SFIS)	18.92±9.36[a] (200)
(Cyclophoshamide)	15	4 (Balb C)	16.20±4.18[b] (200)

a – $p<0.01$; b – $p<0.025$;
c – $p<0.05$ – significant difference vs. control.

significant increase in SCE frequency resulting from exposure to the Sulfosuccinate IO-5, Rokamid MRZ 17 and Rokacet RZG7P2 at any dose.

Significant responses in SCE induction were obtained at the three highest doses (50, 75 and 100 mg/kg) of Roksol TL-7. The mean frequency of SCE per cell was significantly different from that of the control ($p<0.05$). Roksol TL-7 at applied doses increased SCE frequencies from 4.84 to 6.70 SCE/cell producing a slightly positive dose-response relationship.

CONCLUSIONS

The results of the present study indicate that:

1. The all four surface active agents are not detectable through their cytogenetic response in the micronucleus test.

2. Sulfosuccinate IO-5, Rokamid MRZ 17 and Rokacet RZG7P2 are shown to be non-mutagenic in the standard Ames assey and were further tested for the SCE-inducing test which also proved not to be genotoxic.

3. Roksol TL-7 was found to produce a weak mutagenicity in the Ames test and weakly positive results in the sister chromatid exchange assay, thus, this compound possesses slight genotoxic activity.

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