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SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

with

(b) (4)

(Test for mutagenic properties in bacteria)

TIMTIN 770

(b) (4)

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Salmonella/Mammalian-
Microsome Mutagenicity Test

No. of experiment: (b) (4)
Batch: EN EP 102

SUMMARY AND CONCLUSIONS

(b) (4) was tested for mutagenic effects on histidine-auxotrophic mutants of Salmonella typhimurium. The investigations were performed with the following concentrations of the trial substance with and without microsomal activation: 25, 75, 225, 675 and 2025 µg/0.1 ml.

These tests permit the detection of point mutations in bacteria induced by chemical substances. Any mutagenic effects of the substances are demonstrable on comparison of the numbers of bacteria in the treated and control cultures that have undergone back-mutation to histidine-prototrophism. To ensure that mutagenic effects of metabolites of the test substances formed in mammals would also be detected, experiments were performed in which the cultures were additionally treated with an activation mixture (rat liver microsomes and co-factors)[1,2,3].

In the experiments without microsomal activation, comparison of the numbers of back-mutant colonies in the controls and the cultures treated with the various concentrations of (b) (4) revealed a reduction in the colony count due to a growth-inhibiting effect of

the compound at the concentrations of 675 and 2025 µg/0.1 ml respectively.

No evidence of the induction of point mutations by (b) (4) or by the metabolites of the substance formed as a result of microsomal activation was detectable in the strains of S. typhimurium used in these experiments.

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MATERIALS AND METHODS

The bacteria on which the tests were performed were the histidine auxotrophic TA 98, TA 100, TA 1535, and TA 1537 strains of Salmonella typhimurium. Cultures were prepared from frozen stocks, and on the following days the Standard Plate Test was carried out with and without the addition of activation mixture (rat liver microsomes and co-factors)[1,2,3].

The test was performed with the following concentrations of the trial substance with and without microsomal activation: 25, 75, 225, 675, and 2025 g/0.1 ml. The substance was dissolved in acetone. Acetone alone was used for the negative controls (the substances and vehicles used for the positive controls are indicated below). Each Petri dish contained: 1) approx. 20 ml of minimum agar (Agar purified, "Difco" certified, Difco Laboratories, Detroit, Michigan, U.S.A., Art.No.0560, plus salts (Vogel-Bonner Medium E) and glucose), 2) 0.1 ml of the solution of the test substance or the vehicle and 0.1 ml of a bacterial culture (in nutrient broth: Bacto Nutrient Broth dehydrated, Difco Laboratories, Detroit Michigan, U.S.A., Art.No.0003 0.8% plus 0.5% NaCl) in 2.0 ml of soft agar. The soft agar was composed of: 100 ml of 0.6% agar solution (Agar purified, "Difco" certified) with 0.6% NaCl and 10 ml of a solution of l-histidine, 0.5 mM (Fluka, Buchs, Switzerland, Art. No.14400) and +biotin 0.5 mM (Fluka, Buchs, Switzerland, Art. No.53320). In the experiments in which the substance was metabolically activated, 0.5 ml of an activation mixture was added also[2,3]. 1 ml activation mixture contains: 0.3 ml S9 fraction of liver from rats induced with Aroclor 1254 (Analabs), 8 µmoles $MgCl_2$, 33 µmoles KCl, 5 µmoles glucose-6-phosphate, 4 µmoles NADP and 100 µmoles phosphate buffer, pH 7.4.

Positive control experiments were carried out simultaneously with the following substances: 1) for Strain TA 1535: N-methyl-N'-nitro-N-nitrosoguanidine (Fluka, Buchs, Switzerland, Art.

No.68051), 3 and 5 µg/0.1 ml phosphate buffer; 2) for Strain TA 1537: 9(5)aminoacridine hydrochloride monohydrate (Fluka, Buchs, Switzerland, Art.No.06650), 25, 50, and 100 µg/0.1 ml DMSO; 3) for Strain TA 98: daunoblastin (Soc. Farmaceutici Italia, Milan, Italy), 2.5, 5, and 10 µg/0.1 ml phosphate buffer; 4) for Strain TA 100: 4-nitroquinoline-N-oxide (Fluka, Buchs, Switzerland, Art.No.73265), 0.0625, 0.125, and 0.25 µg/0.1 ml phosphate buffer. The activation mixture was tested with Strain TA 1535 and cyclophosphamide, 100 and 250 µg/0.1 ml phosphate buffer.

In the experiments with and without the addition of microsomal activation mixture three Petri dishes were prepared per strain and per group (i.e. per concentration or per control group). In the positive control experiments two Petri dishes were used per strain and per group.

The plates were incubated for about 48 hours at 37°C in darkness.

When the colonies had been counted, the arithmetic mean was calculated. The test substance was considered to be non-mutagenic if the colony count in relation to the negative control was not doubled at any concentration[3].

RESULTS

(see Tables 1 and 2)

In the experiments performed without and with microsomal activation, none of the tested concentrations of (b) (4) caused any increase in the incidence of histidine-prototrophic mutants in comparison with the controls.

After exposure to (b) (4) in the concentrations of 675 and 2025 ug/0.1 ml without microsomal activation, the number of colonies of histidine-prototrophic mutants was reduced as a result of the inhibitory effects of the substance on the growth of the bacteria.

[1]AMES, B.N., F.D. LEE, and W.E. DURSTON (1973), An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens. Proc. Natl. Acad. Sci. USA 70, 782-786.

[2]AMES, B.N., W.E. DURSTON, E. YAMASAKI, and F.D. LEE (1973), Carcinogens are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection. Proc. Natl. Acad. Sci. USA 70, 2281-2285.

[3]AMES, B.N., J. McCANN, and E. YAMASAKI (1975), Methods for Detecting Carcinogens and Mutagens with the Salmonella/

Table 1

Salmonella/Mammalian-Microsome Mutagenicity Test
Experiments without microsomal activation
Number (arithmetic mean) of colonies of
histidine-prototrophic back-mutants

Test substance		Strain of S. typhimurium used			
		TA 98	TA 100	TA 1535	TA 1537
(b) (4)	Control	23	185	15	7
	25 µg/0.1 ml	23	190	21	9
	75 µg/0.1 ml	23	212	14	11
	225 µg/0.1 ml	24	160	5	10
	675 µg/0.1 ml	1	187	8	4
	2025 µg/0.1 ml*	8	90	(34)	3
Positive controls					
Daunoblastin	Control	27			
	2.5 µg/0.1 ml	215			
	5.0 µg/0.1 ml	349			
	10.0 µg/0.1 ml	718			
4-Nitroquinoline-N-oxide	Control		194		
	0.0625 µg/0.1 ml		321		
	0.125 µg/0.1 ml		489		
	0.25 µg/0.1 ml		855		
N-Methyl-N'-nitro-N-nitroso-guanidine	Control			11	
	3 µg/0.1 ml			79	
	5 µg/0.1 mlg			923	
9(5)Aminoacridine hydrochloride	Control				11
	25 µg/0.1 ml				13
	50 µg/0.1 ml				55
	100 µg/0.1 ml				>1150

* At the highest concentration the substance precipitated in soft agar.

() In this value histidine-auxotrophic colonies are included; these arised due to the inhibition effect of the substance on bacterial growth.

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Table 2

Salmonella/Mammalian-Microsome Mutagenicity Test
Experiments with microsomal activation
Number (arithmetic mean) of colonies of
histidine-prototrophic back-mutants

		Strain of S. typhimurium used			
		TA 98	TA 100	TA 1535	TA 1537
Test substance					
(b) (4)	Control	36	140	15	10
	25 µg/0.1 ml	29	180	16	9
	75 µg/0.1 ml	31	157	13	9
	225 µg/0.1 ml	30	178	16	7
	675 µg/0.1 ml	33	168	16	13
	2025 µg/0.1 ml*	17	165	12	7
Positive control of the microsomal activation					
Cyclophosphamide					
23.11.78	Control			15	
	100 µg/0.1 ml			361	
	250 µg/0.1 ml			514	
12.12.78	Control			11	
	100 µg/0.1 ml			195	
	250 µg/0.1 ml			415	

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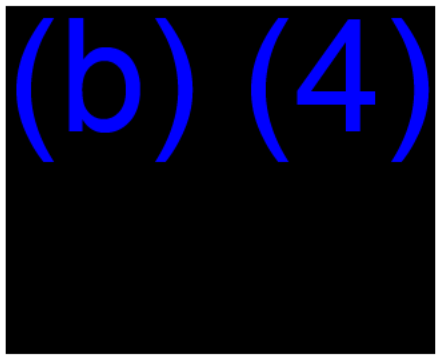
*At the highest concentration the substance precipitated in soft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