

2792
CIBA-GEIGY Corporation
Ardsley, New York 10502-2699
Telephone 914 478 3131

CIBA-GEIGY

January 27, 1987

CONTAINS NO CBI



Document Control Officer (TS-790)
(Attn: Section 8(e) Coordinator)
Information Management Division
Office of Toxic Substances
U. S. Environmental Protection Agency
401 "M" Street, S. W.
Washington, D. C. 20460

CERTIFIED MAIL
RETURN RECEIPT REQUESTED

SANITIZED COPY

RE: 8EHQ-0386-0594S, Ocryltin Ester Mixture;
EPA Requested Information

8EHQ-0287-0594SFLWP
PDCN-88-860000069
89-870000098

Dear Madam/Sir:

CIBA-GEIGY Corporation requests that all information shown in brackets in this letter and in the two enclosed toxicity studies be treated as Confidential Business Information. We enclose a sanitized copy of this letter and of the studies for the public file.

Enclosed is a copy of the positive Ames Test for [] cited on page four, Item 5. (e) of our July 23, 1986 letter. This was requested by Pauline Wagner by telephone about a week ago. We are also enclosing a copy of the negative Ames test cited as Item 5 (d).

The first test (8/26/79) was performed at a concentration of up to 1,215 ug/0.1 ml and was judged negative. The second test was reported on 9/15/83 and used a high concentration of 24,300 ug/0.1 ml. The second test was judged to display a weak mutagenic effect at very high test concentrations.

Very truly yours,

CIBA-GEIGY Corporation

A. Di Battista
A. Di Battista
Manager, Toxic Substances Compliance
Safety, Health & Ecology

ADIB4/01/bt
Attachments

0002

CIBA-GEIGY LIMITED
BASLE, SWITZERLAND

8EHQ-0287-05949
FLWP

Protection of Health
and Environment

CONFIDENTIAL

GD 2.3
Experimental Pathology

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

Test material: TK 10 315 [

~~CONFIDENTIAL~~
]*

Project No.: 830541

* Confidential Information is
Bracketed

Date: September 15, 1983

I N D E X

3

General	Page 2
Summary and Conclusions	Page 3 - 4
Procedure	Page 5 - 6
Results	Page 7
Tables 1-8	Page 8 - 15

GENERAL

Type of study: AT ST O4D MO
Test organisms: S. typhimurium TA 98, TA 100, TA 1535,
TA 1537, TA 1538
Test material: TK 10 315 []
Batch No.: 483/84/85/86/91
Purity: 100%
Stability: To be provided by sponsor
Validity: April 1984

~~CONFIDENTIAL~~

Sponsor: CIBA-GEIGY Limited, Plastics and
Additives Division, Basle, Switzerland
Testing facility: CIBA-GEIGY Limited, Basle, Switzerland
GU 2.3, Experimental Pathology
Laboratories: R-1040.B.42-45
Technical conduct: Mr. Ch. Felix,
Mrs. M. Karimi and Miss D. Weider
Test No.: 830541
Starting date: June 14, 1983
Date of completion: July 5, 1983
Reported and filed: August 1983
Location of archives: CIBA-GEIGY Limited, Basle, Switzerland,
R-1040.K.

PROPERTY OF CIBA-GEIGY LIMITED
CONFIDENTIAL

MAY NOT BE USED, DIVULGED, OR PUBLISHED WITHOUT THE CONSENT OF CIBA-GEIGY LIMITED

SUMMARY AND CONCLUSIONS

~~CONFIDENTIAL~~

TK 10 315 [] was tested for mutagenic effects on histidine-auxotrophic mutants of *Salmonella typhimurium*. The investigations were performed on strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 with the following concentrations of the trial substance without and with microsomal activation: 300, 900, 2700, 8100 and 24,300 $\mu\text{g}/0.1 \text{ ml}$. In order to confirm the results, the experiments were repeated.

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) (lit.1,2,3).

In the experiments performed without microsomal activation treatment with TK 10 315 led to a slight increase in the number of back-mutant colonies of strain TA 100 at the concentrations of 2700 $\mu\text{g}/0.1 \text{ ml}$ and above.

In the experiments performed without microsomal activation on strains TA 1535, TA 1537 and TA 1538, comparison of the number

PROPERTY OF CIBA-GEIGY LIMITED
CONFIDENTIAL

MAY NOT BE USED, DIVULGED, OR PUBLISHED WITHOUT THE CONSENT OF CIBA-GEIGY LIMITED

5

of back-mutant colonies in the controls and the cultures treated with the various concentrations of TK 10 315 revealed a reduction in the colony count due to a growth-inhibiting effect of the compound at the highest concentration.

In the experiments performed with microsomal activation, comparison of the number of back-mutant colonies in the controls and the cultures treated with the various concentrations of TK 10 315 revealed no marked deviations.

TK 10 315 thus displayed an albeit weak mutagenic effect in this test system.



Study director:

(E. Deperade)

Date: September 14, 1983



Report reviewed
and approved by:

(Dr. P. Arni)

(Senior scientist,
Experimental Pathology)

Date: September 15, 1983

PROCEDURE

The tests were carried out in accordance with the method described by AMES et al. (lit.1,2,3).

The bacteria on which the tests were performed were the following histidine-auxotrophic strains of *Salmonella typhimurium*: TA 98, TA 100, TA 1535, TA 1537 and TA 1538 (origin: Prof. B. Ames, Berkeley, U.S.A.).

The tests were performed with the following concentrations of the trial substance without and with microsomal activation: 300, 900, 2700, 8100 and 24,300 µg/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, Difco Laboratories, Detroit, Michigan, U.S.A., 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, Difco Laboratories, Detroit, Michigan, U.S.A., 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 with 0.6% NaCl and 10 ml of a solution of l-histidine, 0.5 mM (Fluka, Buchs, Switzerland) and +biotin 0.5 mM (Fluka, Buchs, Switzerland). In the experiments in which the substance was metabolically activated, 0.5 ml of an activation mixture was added also (lit.1,2,3). 1 ml activation mixture contained: 0.3 ml S9 fraction of liver from rats (Tif:-RAIf(SPF)) induced with Aroclor 1254 (Analabs., Inc., North Haven, Connecticut, U.S.A.) and 0.7 ml of a solution of co-factors.

Positive control experiments were carried out simultaneously with the following substances: 1) for strain TA 98: daunorubi-

PROPERTY OF CIBA-GEIGY LIMITED
CONFIDENTIAL

MAY NOT BE USED, DIVULGED, OR PUBLISHED WITHOUT THE CONSENT OF CIBA-GEIGY LIMITED

7
cin-HCl (DAUNOBLASTIN[®], Farmitalia, Montedison Farmaceutica GmbH, Freiburg i.Br., Germany), 5 and 10 µg/0.1 ml phosphate buffer; 2) for strain TA 100: 4-nitroquinoline-N-oxide (Fluka, Buchs, Switzerland), 0.125 and 0.25 µg/0.1 ml phosphate buffer; 3) for strain TA 1535: N-methyl-N'-nitro-N-nitrosoguanidine (Serva, Heidelberg, Germany), 3 and 5 µg/0.1 ml phosphate buffer; 4) for strain TA 1537: 9(5)aminoacridine hydrochloride monohydrate (Fluka, Buchs, Switzerland), 50 and 100 µg/0.1 ml DMSO; 5) for strain TA 1538: 2-nitrofluorene (Fluka, Buchs, Switzerland), 5 and 10 µg/0.1 ml DMSO. The activation mixture was tested with strain TA 1535 and cyclophosphamide (ENDOXAN ASTA[®], Asta-Werke, Bielefeld, Germany), 250 µg/0.1 ml phosphate buffer. In the repeat experiment, the investigation with strain TA 100 was carried out separately. In this case the activation mixture was tested with this strain and with 2-aminoanthracene (EGA Chemie, Steinheim, Germany), 5.0 µg/0.1 ml DMSO.

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

The plates were incubated for about 48 hours at $37 \pm 1.5^{\circ}\text{C}$ in darkness.

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

PROPERTY OF CIBA-GEIGY LIMITED
CONFIDENTIAL

MAY NOT BE USED, DIVULGED, OR PUBLISHED WITHOUT THE CONSENT OF CIBA-GEIGY LIMITED

RESULTS

(see Tables 1 to 8)

In the experiments carried out without microsomal activation, treatment with TK 10 315 led to a slight increase in the number of back-mutant colonies of strain TA 100 at the concentrations of 2700 ug/0.1 ml and above.

A growth-inhibiting effect of the compound was observed in the experiments without activation on strains TA 1535, TA 1537 and TA 1538 at the concentration of 24,300 ug/0.1 ml.

The slight increase in the number of back-mutant colonies observed in the first experiment with activation on strain TA 1537 at the concentrations of 300 and 2700 ug/0.1 ml is attributed to fluctuations in the rate of spontaneously occurring back-mutants.

At the concentrations of 8100 and 24,300 ug, 0.1 ml the substance precipitated in soft agar.

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/Mammalian-Microsome Mutagenicity Test. Mutation Res. 31, 347-364.

TABLE 4

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST
 EXPERIMENTS WITHOUT MICROSOMAL ACTIVATION
 NUMBER OF BACK-MUTANT COLONIES PER PLATE (ARITHMETIC MEAN)

9

No. of experiment: 830541 Test substance: TK 10 315

Date: June 16, 1983

STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538
Control	24	180	16	6	12
300 µg/0.1 ml	26	172	11	7	9
900 µg/0.1 ml	26	181	11	6	11
2700 µg/0.1 ml	21	251	11	6	7
8100 µg/0.1 ml	25	387	9	5	5
24300 µg/0.1 ml	23	428	5	1	7
Positive controls					
daunorubicin-HCl					
Control	24				
5 µg/0.1 ml	286				
10 µg/0.1 ml	993				
4-nitroquinoline-N-oxide					
Control		167			
0.125 µg/0.1 ml		659			
0.25 µg/0.1 ml		1046			
N-methyl-N'-nitro-N-nitrosoguanidine					
Control			13		
3 µg/0.1 ml			335		
5 µg/0.1 ml			1937		
9(5) aminoacridine-hydrochloride					
Control				9	
50 µg/0.1 ml				49	
100 µg/0.1 ml				507	
2-nitrofluorene					
Control					11
5 µg/0.1 ml					802
10 µg/0.1 ml					1144

PROPERTY OF CIBA-GEIGY LIMITED

CONFIDENTIAL

MAY NOT BE USED, DIVULGED, OR PUBLISHED WITHOUT THE CONSENT OF CIBA-GEIGY LIMITED

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST
EXPERIMENTS WITH MICROSOMAL ACTIVATION
NUMBER OF BACK-MUTANT COLONIES PER PLATE (ARITHMETIC MEAN)

No. of experiment: 830541 Test substance: TK 10 315

Date: June 16, 1983

STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538
Control	34	139	14	7	25
300 ug/0.1 ml	46	154	11	14	24
900 ug/0.1 ml	42	177	15	12	27
2700 ug/0.1 ml	37	147	8	15	28
8100 ug/0.1 ml	36	173	14	9	21
24300 ug/0.1 ml	33	183	14	7	29

Positive control of the
microsomal activation

cyclophosphamide

Control	14
250 ug/0.1 ml	675

2-aminoanthracene

Control	
5 ug/0.1 ml	

PROPERTY OF CIBA-GEIGY LIMITED
CONFIDENTIAL

MAY NOT BE USED, DIVULGED, OR PUBLISHED WITHOUT THE CONSENT OF CIBA-GEIGY LIMITED

TABLE 1

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST
 EXPERIMENTS WITHOUT MICROSOMAL ACTIVATION: NUMBER OF BACK-MUTANT
 COLONIES PER PLATE, ARITHMETIC MEAN AND STANDARD DEVIATION ()

No. of experiment: 830541 Test substance: TK 10 315

Date: June 16, 1983

STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538	
Control	26 28 17 24(6)	187 176 176 180(6)	16 16 16 16(0)	8 7 4 6(2)	14 16 7 12(5)	
300 µg/0.1 ml	20 29 30 26(6)	170 182 165 172(9)	9 12 13 11(2)	5 8 8 7(2)	8 12 7 9(3)	
900 µg/0.1 ml	21 27 29 26(4)	174 182 182 181(7)	12 8 12 11(4)	5 7 7 6(1)	9 13 12 11(2)	
2700 µg/0.1 ml	18 20 26 21(4)	240 229 283 251(29)	16 7 9 11(5)	5 5 9 6(2)	7 6 9 7(2)	
8100 µg/0.1 ml	28 27 20 25(4)	402 384 375 387(14)	9 12 6 9(3)	7 4 5 5(2)	7 4 6 6(2)	
24300 µg/0.1 ml	20 21 29 23(5)	429 343 513 428(85)	5 3 6 5(2)	0 2 2 1(1)	0 3 18 7(10)	
Positive controls	Control	18 27 26 24(5)	185 152 165 167(17)	12 13 13 13(1)	12 9 5 9(4)	12 12 8 11(2)
Concentration A	230 230 399 286(98)	662 663 651 659(7)	319 --- 351 335(23)	50 42 54 49(6)	663 853 889 802(121)	
B	885 1013 1082 993(100)	1044 1116 977 1046(70)	1736 1737 2337 1897(347)	495 388 637 507(125)	1231 984 1217 1144(139)	

0012

TABLE 4

SALMONELLA/WHITLIPAN-MICROSOME MUTAGENICITY TEST
 EXPERIMENTS WITH MICROSOmal ACTIVATION: NUMBER OF BACK-MUTANT
 COLONIES PER PLATE, ARITHMETIC MEAN AND STANDARD DEVIATION ()

No. of experiment: 830541 Test substance: TK 10 315

Date: June 16, 1983

STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538
Control	41 28 36 34 (8)	126 163 127 139 (21)	17 14 12 14 (3)	9 5 7 7 (2)	21 26 27 25 (3)
300 µg/0.1 ml	48 48 42 46 (3)	150 144 158 154 (9)	6 14 12 11 (4)	15 13 13 14 (1)	24 24 25 24 (1)
900 µg/0.1 ml	40 44 42 40 (2)	164 151 107 117 (26)	13 12 21 15 (5)	14 8 15 12 (4)	28 27 27 27 (1)
3000 µg/0.1 ml	31 32 30 27 (5)	129 135 126 147 (26)	5 9 9 8 (2)	14 17 15 15 (2)	28 27 29 28 (1)
8100 µg/0.1 ml	29 30 28 26 (12)	168 171 151 173 (21)	9 17 15 14 (4)	14 5 8 9 (5)	20 25 19 21 (3)
14300 µg/0.1 ml	28 43 28 33 (9)	165 166 150 183 (16)	16 12 13 14 (2)	8 6 7 7 (1)	27 25 36 29 (6)

Positive control of the
 microsomal activation

Control	8 20 14 14 (6)
Concentration A	705 737 582 675 (82)

PROPERTY OF CIBA-GEIGY LIMITED
 CONFIDENTIAL

MAY NOT BE USED, DIVULGED, OR PUBLISHED WITHOUT THE CONSENT OF CIBA-GEIGY LIMITED

0.013

TABLE 3

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST
 EXPERIMENTS WITHOUT MICROSOMAL ACTIVATION
 NUMBER OF BACK-MUTANT COLONIES PER PLATE (ARITHMETIC MEAN)

No. of experiment: 830541 Test substance: TK 10 315

13 Date: June 24 and July 05, 1983

STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538
Control	31	127	15	9	11
300 µg/0.1 ml	37	145	12	5	15
900 µg/0.1 ml	27	161	18	7	14
2700 µg/0.1 ml	29	188	12	5	8
8100 µg/0.1 ml	27	286	12	5	7
24300 µg/0.1 ml	31	420	11	3	7
Positive controls					
daunorubicin-HCl					
Control	33				
5 µg/0.1 ml	440				
10 µg/0.1 ml	978				
4-nitroquinoline-N-oxide					
Control		130			
0.125 µg/0.1 ml		652			
0.25 µg/0.1 ml		961			
N-methyl-N'-nitro-N-nitrosoguanidine					
Control			15		
3 µg/0.1 ml			842		
5 µg/0.1 ml			1575		
9(5) aminoacridine-hydrochloride					
Control				7	
50 µg/0.1 ml				46	
100 µg/0.1 ml				612	
2-nitrofluorene					
Control					14
5 µg/0.1 ml					956
10 µg/0.1 ml					1189

PROPERTY OF CIBA-GEIGY LIMITED
 CONFIDENTIAL

MAY NOT BE USED, DIVULGED, OR PUBLISHED WITHOUT THE CONSENT OF CIBA-GEIGY LIMITED

INHIBITION OF AMMOXICILLIN-MICROSOME MUTAGENICITY TEST
 EXPERIMENTS WITH MICROSOXIMAL ACTIVATION
 NUMBER OF BACK-MUTANT COLONIES PER PLATE (ARITHMETIC MEAN)

No. of experiment: 8305-1 Test substance: TM 10 315

Date: June 24 and July 05, 1983

STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538
Control	47	132	14	18	32
300 ug/0.1 ml	57	125	15	14	29
900 ug/0.1 ml	55	9	22	16	35
2700 ug/0.1 ml	53	139	17	14	21
8100 ug/0.1 ml	44	112	16	9	21
14300 ug/0.1 ml	41	116	9	7	20

Positive control of the
microsomal activation:

cyclophosphamide

Control	19
250 ug/0.1 ml	500

2-aminocanthracene

Control	113
5 ug/0.1 ml	191

PROPERTY OF CIBA-GEIGY LIMITED
CONFIDENTIAL

MAY NOT BE USED, DIVULGED, OR PUBLISHED WITHOUT THE CONSENT OF CIBA-GEIGY LIMITED

TABLE 7

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST
 EXPERIMENTS WITHOUT MICROSOMAL ACTIVATION: NUMBER OF BACK-MUTANT
 COLONIES PER PLATE, ARITHMETIC MEAN AND STANDARD DEVIATION ()

No. of experiment: 830541 Test substance: TK 10 315

Date: June 24 and July 05, 1983

STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538
Control	30	132	14	6	8
	37	123	16	7	17
	27	127	16	13	8
	31(5)	127(5)	15(1)	9(4)	11(5)
	32	148	7	6	14
300 µg/0.1 ml	41	136	13	6	13
	38	152	16	3	18
	37(5)	145(8)	12(5)	5(2)	15(3)
	31	156	16	8	12
	19	165	21	4	16
900 µg/0.1 ml	32	162	17	8	14
	27(7)	161(5)	18(3)	7(2)	14(2)
	29	198	9	4	4
	27	180	14	3	8
	32	185	13	7	12
2700 µg/0.1 ml	29(3)	188(9)	12(3)	5(2)	8(4)
	31	325	9	3	12
	30	280	13	4	3
	20	253	13	8	6
	27(6)	286(36)	12(2)	5(3)	7(5)
8100 µg/0.1 ml	33	402	9	3	6
	30	438	13	2	7
	31	421	12	3	7
	31(2)	420(18)	11(2)	3(1)	7(2)
	Positive controls				
Control	38	127	17	8	16
	28	123	20	5	13
	33	129	9	8	14
	33(5)	130(7)	15(6)	7(2)	14(2)
	Concentration A	482	631	837	56
B	374	663	602	31	963
	463	661	1088	51	760
	440(58)	652(18)	842(243)	46(13)	956(193)
	976	1002	1514	687	1207
	940	987	1502	484	1108
B	1017	895	1609	679	1253
	978(39)	961(58)	1575(53)	617(115)	1189(74)

PROPERTY OF CIBA-GEIGY LIMITED

CONFIDENTIAL

MAY NOT BE USED, DIVULGED, OR PUBLISHED WITHOUT THE CONSENT OF CIBA-GEIGY LIMITED

TABLE 8

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST
EXPERIMENTS WITH MICROSOMAL ACTIVATION: NUMBER OF BACK-MUTANT
COLONIES PER PLATE, ARITHMETIC MEAN AND STANDARD DEVIATION ()

No. of experiment: 830541 Test substance: TK 10 315

Date: June 24 and July 05, 1983

STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538
Control	45 43 54	116 157 123	19 8 15	17 17 20	29 41 27
300 µg/0.1 ml	47(6) 55 50 55	132(22) 139 116 120	14(6) 21 13 12	18(2) 20 7 15	32(8) 33 26 27
900 µg/0.1 ml	53(3) 67 45 52	125(12) 103 149 135	15(5) 16 20 30	14(7) 21 13 14	29(4) 29 36 39
2700 µg/0.1 ml	55(11) 69 36 55	129(24) 157 121 139	22(7) 20 18 12	16(4) 17 19 6	35(5) 21 16 27
8100 µg/0.1 ml	53(17) 52 43 38	139(18) 92 122 121	17(4) 12 24 13	14(7) 6 5 15	21(6) 19 19 26
24300 µg/0.1 ml	44(7) 36 42 53	112(17) 108 117 123	16(7) 9 8 9	9(6) 7 6 7	21(4) 26 15 19
	44(9)	116(8)	9(1)	7(1)	20(6)
Positive control of the microsomal activation					
Control		109 120 110	25 18 15		
Concentration A		113(6)	19(5)		
		156 199 217	609 517 524		
		191(31)	550(51)		

PROPERTY OF CIBA-GEIGY LIMITED
CONFIDENTIAL

MAY NOT BE USED, DIVULGED, OR PUBLISHED WITHOUT THE CONSENT OF CIBA-GEIGY LIMITED

Verteiler:

17

HH.	Dr. P. Arni	(1x)
	E. Deparade	(1x)
	Prof. D. Müller	(2x)
	Dr. A. von Schulthess	(2x)

Basle, Switzerland

19

CONTAINS NO OPI

8EHQ-0287-05945
FLWP.

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

with

TK 10 315

(Test for mutagenic properties in bacteria)

[~~CONFIDENTIAL~~]*

* Confidential Information is Bracketed

GU 2.3

August 28, 1979

SUMMARY AND CONCLUSIONS

TK 10 315 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: 15, 45, 135, 405 and 1215 $\mu\text{g}/0.1 \text{ ml}$.

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 substance 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 performed without and with microsomal activation comparison of the number of back-mutant colonies in the controls and the cultures treated with the various concentrations of TK 10 315 revealed no marked deviations.

No evidence of the induction of point mutations by TK 10 315 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.

21

Study director:



(Dr. P. Arni)

Date: August 27, 1979

Report reviewed and approved by:



(Prof. Dr. D. Müller)

(Head of Experimental Pathology)

Date: August 28, 1979

CIBA-GEIGY Limited
Basle, Switzerland
Protection of Health
and Environment
Toxicology

The tests were carried out in accordance with the method described by AMES et al.^{1,2,3}.

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*.

The test was performed with the following concentrations of the trial substance without and with microsomal activation: 15, 45, 135, 405 and 1215 $\mu\text{g}/0.1$ ml. The substance was dissolved in acetone. Acetone alone was used for the negative controls. In the experiments in which the substance was metabolically activated, 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 and 0.7 ml of a solution of co-factors.

Positive control experiments were carried out simultaneously with the following substances: 1) for Strain TA 98: daunorubicin-HCl (DAUNOBLASTIN[®]), 5 and 10 $\mu\text{g}/0.1$ ml phosphate buffer; 2) for Strain TA 100: 4-nitroquinoline-N-oxide, 0.125 and 0.25 $\mu\text{g}/0.1$ ml phosphate buffer; 3) for Strain TA 1535: N-methyl-N'-nitro-N-nitrosoguanidine, 3 and 5 $\mu\text{g}/0.1$ ml phosphate buffer; 4) for Strain TA 1537: 9(5)aminoacridine hydrochloride monohydrate, 50 and 100 $\mu\text{g}/0.1$ ml DMSO. The activation mixture was tested with Strain TA 1535 and cyclophosphamide (ENDOXAN-ASTA[®]), 250 $\mu\text{g}/0.1$ 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).

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

When the colonies had been counted, the arithmetic mean was calculated. A test substance is generally considered to be non-mutagenic if the colony count in relation to the negative control is not doubled at any concentration³.

23

(see Tables 1 and 2)

In the experiments performed without and with microsomal activation comparison of the number of histidine-prototrophic mutants in the controls and after treatment with TK 10 315 revealed no marked differences.

¹AMES, 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.

²AMES, 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.

³AMES, B.N., J. McCANN, and E. YAMASAKI (1975), Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test. Mut. Res. 31, 347-364.

Salmonella/Mammalian-Microsome Mutagenicity Test

Experiments without microsomal activation

Number (arithmetic mean) of colonies of
histidine-prototrophic back-mutants

25

<u>Test substance</u>	<u>Strain of S. typhimurium used</u>			
	TA 98	TA 100	TA 1535	TA 1537
TK 10 315				
Control	31	174	8	4
15 µg/O.1 ml	24	184	9	3
45 µg/O.1 ml	22	195	9	4
135 µg/O.1 ml	24	177	12	3
405 µg/O.1 ml	27	209	11	3
1215 µg/O.1 ml*	32	217	8	4
 <u>Positive controls</u>				
Daunorubicin-HCl				
Control	26			
5.0 µg/O.1 ml	888			
10.0 µg/O.1 ml	~1250			
 4-Nitroquinoline-N-oxide				
Control		160		
0.125 µg/O.1 ml		760		
0.25 µg/O.1 ml		~1270		
 N-Methyl-N'-nitro-N-nitrosoguanidine				
Control			9	
3 µg/O.1 ml			~1280	
5 µg/O.1 ml			~1890	
 9(5)Aminoacridine hydrochloride				
Control				6
50 µg/O.1 ml				115
100 µg/O.1 ml				~1150

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

Salmonella/Mammalian-Microsome Mutagenicity Test

experiments with microsomal activation

Number (arithmetic mean) of colonies of

histidine-prototrophic back-mutants

<u>Test substance</u>		<u>Strain of S. typhimurium used</u>			
		TA 98	TA 100	TA 1535	TA 1537
TK 10 315	Control	32	187	13	7
	15 ug/O.1 ml	32	189	14	7
	45 ug/O.1 ml	39	180	11	3
	135 ug/O.1 ml	39	166	10	2
	405 ug/O.1 ml	41	177	8	3
	1215 ug/O.1 ml*	42	175	13	2
<u>Positive control of the microsomal activation</u>					
Cyclophosphamide	Control			9	
	250 ug/O.1 ml			679	

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

Verteiler:

HH. Dr. P. Arni (2x)

21 Prof. R. Hess (1x)

Dr. R. Leimgruber (2x)

Prof. D. Müller (2x)

M
2/4/87

...

RECEIVED

0 0 3 4