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Pharma Development Central Toxicology

Study Title

Dodigen 4022

STUDY OF THE MUTAGENIC POTENTIAL IN STRAINS OF SALMONELLA TYPHIMURIUM (AMES TEST) AND ESCHERICHIA COLI

Author

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July 10th, 1992

Performing laboratory

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Laboratory Project ID:

Study No. 92.0336

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STATEMENT OF COMPLIANCE

To the best of my knowledge and belief, this study was conducted in compliance with Good Laboratory Practice regulations. No unforeseen circumstances were observed which might have affected the quality or integrity of the study.

Study Director [redacted]

Testing facility management: [redacted] 27 July 1993

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Quality Assurance Statement

Hoechst Aktiengesellschaft
Quality Assurance(GLP)

09.07.1992

Title : Dodigen 4022
STUDY OF THE MUTAGENIC POTENTIAL
IN STRAINS OF SALMONELLA TYPHIMURIUM
(AMES TEST) AND ESCHERICHIA COLI

STUDY NO. : 92.0336

This study was periodically inspected and properly signed records of these inspections were submitted to testing facility management and the study director as shown below :

Inspection	Report
04.06.1992	05.06.1992
10.06.1992	10.06.1992
03.07.1992	03.07.1992
09.07.1992	09.07.1992

[redacted]

09.07.1992

[redacted]GLP)

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CONTENTS

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1. SUMMARY

Dodigen 4022 was tested for mutagenicity with the strains TA 100, TA 1535, TA 1537, TA 1538, TA 98 of Salmonella typhimurium and Escherichia coli WP2uvrA.

The mutagenicity studies were conducted in the absence and in the presence of a metabolizing system derived from rat liver homogenate. A dose range of 7 different doses from 4 microgram/plate to 10 000 microgram/plate was used.

Control plates without mutagen showed that the number of spontaneous revertant colonies was similiar to that described in the literature. All the positive control compounds gave the expected increase in the number of revertant colonies.

Toxicity: The test compound proved to be not toxic to the bacterial strains. 5 000 or 10 000 microgram/plate was chosen as top dose level for the mutagenicity study.

Mutagenicity: In the absence of the metabolic activation system the test compound did not show a dose dependent increase in the number of revertants in any of the bacterial strains. Also in the presence of a metabolic activation system, treatment of the cells with Dodigen 4022 did not result in relevant increases in the number of revertant colonies.

Summarizing, it can be stated that Dodigen 4022 is not mutagenic in these bacterial test systems either with or without exogenous metabolic activation at the dose levels investigated.

2. INTRODUCTION

This report describes experiments performed in a short term test using the procedure of the Salmonella / mammalian-microsome-mutagenicity test (Ames Test) (1.2) to assess the mutagenic potential of the test-material in amino acid-dependent strains of Salmonella typhimurium and a strain of Escherichia coli described by Green (3). By the use of liver homogenate the test takes into account the mammalian metabolism of the compound to be tested. The requirement for metabolic activation was investigated by incorporating into the test an activation system by nicotinamide-adenin dinucleotide phosphate ($NADP^+$)-cytochrome P_{450} dependent mixed function oxidase enzymes of the liver. The 9000 g supernatant of rat liver homogenate has been shown to be very useful in metabolic activation of foreign compounds. The animals were pretreated with Aroclor 1254 as an inducer of several drug metabolizing enzymes (4).

In the Ames test with Salmonella typhimurium strains the effect of the test compound upon the number of back mutations to histidine prototrophy using histidine auxotrophic mutants is investigated. Using Escherichia coli WP2uvrA, a tryptophan dependent auxotroph strain, mutagenicity is based on reversion to tryptophan independence. The strains TA 100 and TA 1535 were originally derived by a substitution mutation, the strains TA 1537, TA 1538 and TA 98 by frame shift mutations from histidine prototrophic bacteria. All five Salmonella strains are deficient in the complete structure of their lipopolysaccharide layer and in DNA excision repair system (2). TA 98 and TA 100 possess a modified postreplication DNA repair system which frequently causes an increase in the rate of mutations (5). Strain WP2uvrA carries a defect in one of the genes for tryptophan biosynthesis and is deficient in the uvrA system of DNA repair. The reversion can be induced by a base change (substitution).

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3. GENERAL

Study-No.	:	92.0336
Test compound	:	Dodigen 4022
Ordered by	:	DI Hoechst AG, MTH-Entwicklung TVS, Herr [redacted]
Test system	:	Point mutation assay with bacteria
Test organisms		
Salmonella typhimurium	:	TA 100, TA 1535, TA 1537, TA 1538 and TA 98
Escherichia coli	:	WP2uvrA
Initiation of the study	:	June 03rd, 1992
Termination of the study	:	June 19th, 1992

R e s p o n s i b i l i t y

Head of Toxicology	:	[redacted]
Head of Genetic Toxicology	:	
Quality assurance unit	:	
Testing facilities and archives	:	Pharma Development Central Toxicology HOECHST AKTIENGESELLSCHAFT Post box 80 03 20 6230 Frankfurt 80

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4. MATERIAL AND METHODS

4.1. Test compound

Name	:	Dodigen 4022
Chemical nomenclature	:	Trimethyl-ethoxypolyoxypropylammonium-chloride
Purity	:	82 %
Certificate of analysis	:	No.04701, dated August 20th, 1991, Analytisches Laboratorium, Dr. [redacted]
Storage stability	:	stable for 2 years at 0 °C, dated August 20th, 1991, Dr. [redacted]
Stability in solvent	:	proved for 4 hours, dated December 04th, 1991, Analytisches Laboratorium, Dr. [redacted]
Appearance	:	yellowish viscous turbid liquid
Boiling point	:	> 250 °C
Molecular weight	:	approx. 600 g/mol
Specific gravity	:	approx. 1.01 - 1.03 g/cm^3
Vapor pressure	:	approx. < 10^{-3} mbar at 20 °C
pH - Value in water	:	approx 6.5
Batch No.	:	E 061 59 865
Date of submission	:	April 09th, 1992
Storage conditions	:	dark at approx. - 10 °C in a deep-freezer
Concentration of stock solution	:	10 %

At the day of the experiment the test substance was dissolved in Aqua bidest. at appropriate concentrations.

4.2 Preparation and storage of a liver homogenate fraction ("S-9")

Male Sprague Dawley rats (200 - 300 g) receive a single intraperitoneal injection of Aroclor 1254 (500 mg/kg bodyweight) 5 days before sacrifice. Preparation is performed at approx. 0 to 4 °C using cold sterile solution and glassware. The livers from at least 5 - 6 animals are removed and pooled, washed in 150 mM KCl (approx. 1 ml/g wet livers). The washed livers are cut into small pieces and homogenized in three volumes of KCl. The homogenate is centrifuged at approx. 9000 g for 10 minutes. The supernatant is the S-9 fraction. It is divided into small portions, rapidly frozen and storage at approx. -80 °C for not longer than six months.

4.3 Preparation of S-9 Mix

Sufficient S-9 fraction is thawed immediately before each test at room temperature. One volume of S-9 fraction is mixed with 9 volumes of the S-9 cofactor solution and kept on ice until used. This preparation is termed S-9 Mix. The concentrations of the different compounds in the S-9 Mix are:

8 mM $MgCl_2$
33 mM KCl
5 mM glucose-6-phosphate
4 mM $NADP^+$
100 mM phosphate buffer pH 7.4

4.4 Bacteria

Bacteria are grown overnight in nutrient broth (25 g Oxoid Nutrient Broth No. 2 /liter) at approx. 37 °C. The suitable amount of bacteria in the cell suspension is checked by nephelometry. For inoculation, stock cultures which are stored at approx. -80 °C, are used. The compound is tested with the strains Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537 and TA 1538 and E. coli WP2uvrA. Identification of the different bacterial strains is performed periodically and all criteria for a valid assay were achieved as described (2,3).

4.5 Toxicity experiments and dose range finding

The first experiment was performed with all tester strains using three plates per dose to get information on mutagenicity and toxicity for calculation of an appropriate dose range. A reduced rate of spontaneously occuring colonies as well as visible thinning of the bacterial lawn were used as indicator for toxicity. Thinning of the bacterial lawn was evaluated microscopically.

In combination with the second experiment, toxicity testing was performed as follows: 0.1 ml of the different dilutions of the test compound were thoroughly mixed with 0.1 ml of 10^{-6} dilution of the overnight culture of TA 100 and plate with histidine and biotin rich top agar (3 plates per dose). The solvent control is compared with the number of colonies per plate in the presence of the test compound. Results are given as a ratio of these values (= surviving fraction).

4.6 Mutagenicity test

Top agar is prepared for the Salmonella strains by mixing 100 ml agar (0.6 % agar, 0.5 % NaCl) with 10 ml of a 0.5 mM histidine-biotin solution. With E. coli histidine is replaced by tryptophan (2.5 ml, 0.5 mM). The following ingredients are added (in order) to 2 ml of molten top agar at approx. 45 °C:

0.1 ml of an overnight nutrient broth culture of the bacterial tester strain
0.1 ml test compound solution
0.5 ml S-9 Mix (if required) or buffer

After mixing, the liquid is poured into a petridish with minimal agar (1.5 % agar, Vogel-Bonner E medium with 2 % glucose). After incubation for approximally 48 hours at approx. 37 °C in the dark, colonies (his^+ revertants) are counted.

Two independent experiments were performed.

4.7 Positive controls

Positive control plates were included for each strain. The following substances were used as positive controls.

a) without metabolic activation:

Sodium-azide: TA 100, TA 1535
9-Aminoacridine: TA 1537
2-Nitrofluorene: TA 98, TA 1538
N-Methyl-N-nitro-N-nitrosoguanidine (MNNG): WP2uvrA

b) with metabolic activation:

2-Aminoanthracene: TA 98, TA 100, TA 1535, TA 1537, TA 1538, WP2uvrA

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4.8. Criteria for a positive reponse

A test article is classified mutagenic if either of the following conditions is achieved:

- a test article produces at least a 2-fold increase in the mean number of revertants per plate of at least one of the tester strains over the mean number of revertants per plate of the appropriate vehicle control at complete bacterial background lawn
- a test article induces a dose-related increase in the mean number of revertants per plate of at least one of the tester strains over the mean number of revertants per plate of the appropriate vehicle control in at least two to three concentrations of the test article at complete bacterial background lawn.

- The test results must be reproducible.

5. RESULTS

Dodigen 4022 was tested for mutagenicity with Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 and E. coli WP2uvrA in the absence and presence of a metabolic activation system. The results obtained with the test material and positive control compounds are presented in table 1 to 16a. The number of colonies per plate with each strain as well as mean values of 3 or 4 plates, corrected to the next whole number are given.

5.1 Sterility checks and control plates

Sterility of S-9 Mix and the test compound were indicated by the absence of contamination on the test material and S-9 Mix sterility check plates. Control plates (background control and positive controls) gave the expected number of colonies.

5.2 Toxicity test

The test compound was tested at doses of 4 to 10 000 microgram/plate (table 1 - 6) and proved to be not toxic to the bacterial strains.

For mutagenicity testing the top dose level for each bacterial strain was selected on the basis of the preliminary test results.

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5.3 Mutagenicity test with Dodigen 4022

The test compound did not cause a significant increase in the number of revertant colonies with any of the tester strains in the absence of S-9 Mix. In the presence of S-9 Mix a slight increase in the number of revertants was observed only in TA 98 at the highest tested concentration (table 5). This increase was not reproducible in a second and third independent experiment (table 11 and 13). No dose dependent effect was obtained in any of the tester strains with or without S-9 Mix (table 7 - 12).

It is concluded that the test substance is not mutagenic in these bacterial test systems either in the absence or in the presence of an exogenous metabolizing system.

This test was performed according to the methods described. No unforeseen circumstances were observed which have affected the quality and integrity of this study.

This study was conducted in compliance with the principles of Good Laboratory Practice.

[redacted]

Quality assurance unit
08.07.92

Pharma Development Central Toxicology
HOECHST AKTIENGESELLSCHAFT

[redacted] July 10, 1992
Study Director

[redacted] 9 July 1992
Head of Toxicology

6. TABLES

First experiment

Table 1: Mutagenicity experiment with Dodigen 4022 with and without metabolic activation

TA 100

Number of revertant colonies per plate and mean values using Salmonella typhimurium strain TA 100

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	204	214	182	217
4	-	180	170	202	167
20	-	181	165	186	193
100	-	194	186	186	210
500	-	223	229	190	251
2500	-	242	239	252	234
10000	-	243	206	327	195
0	+	226	222	224	231
4	+	192	171	197	208
20	+	205	224	193	198
100	+	212	211	199	226
500	+	213	199	225	216
2500	+	237	217	258	237
10000	+	256	256	261	252

Compound dissolved in 100 microliter Aqua bidest.

\- : absence
\+ : presence

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First experiment

Table 2: Mutagenicity experiment with Dodigen 4022 with and without metabolic activation

TA 1535

Number of revertant colonies per plate and mean values using Salmonella typhimurium strain TA 1535

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	11	8	9	17
4	-	13	12	16	11
20	-	11	10	13	10
100	-	14	10	12	20
500	-	9	10	6	11
2500	-	10	12	6	12
10000	-	15	21	10	13
0	+	13	12	13	15
4	+	12	15	11	10
20	+	11	10	12	10
100	+	10	9	13	9
500	+	15	9	18	17
2500	+	12	11	12	12
10000	+	10	9	12	9

Compound dissolved in 100 microliter Aqua bidest.

\- : absence
\+ : presence

TA 1537

Number of revertant colonies per plate and mean values
using Salmonella typhimurium strain TA 1537

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	10	10	12	7
4	-	6	6	6	6
20	-	6	8	5	6
100	-	9	6	10	12
500	-	10	12	9	10
2500	-	9	9	13	6
10000	-	11	10	14	10
0	+	6	6	7	4
4	+	6	6	8	5
20	+	6	8	6	3
100	+	10	4	9	18
500	+	10	9	12	8
2500	+	9	8	8	12
10000	+	10	12	9	10

Compound dissolved in 100 microliter Aqua bidest.

\- : absence
\+ : presence

First experiment

Table 4: Mutagenicity experiment with Dodigen 4022 with and without metabolic activation

TA 1538

Number of revertant colonies per plate and mean values using Salmonella typhimurium strain TA 1538

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	18	20	13	20
4	-	15	17	13	16
20	-	15	19	14	12
100	-	17	17	21	14
500	-	17	16	19	17
2500	-	20	24	15	21
10000	-	30	36	30	24
0	+	17	15	13	22
4	+	27	26	30	24
20	+	18	13	24	17
100	+	18	9	24	21
500	+	20	15	26	18
2500	+	23	16	23	31
10000	+	29	28	28	31

Compound dissolved in 100 microliter Aqua bidest.

\- : absence
\+ : presence

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First experiment

Table 5: Mutagenicity experiment with Dodigen 4022 with and without metabolic activation

TA 98

Number of revertant colonies per plate and mean values using Salmonella typhimurium strain TA 98

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	21	21	20	22
4	-	22	27	18	20
20	-	23	23	19	26
100	-	26	20	30	28
500	-	21	19	19	26
2500	-	34	34	29	38
10000	-	27	22	37	21
0	+	21	24	22	18
4	+	27	24	26	32
20	+	27	25	26	31
100	+	34	24	37	41
500	+	33	34	33	31
2500	+	37	39	38	35
10000	+	43	46	48	34

Compound dissolved in 100 microliter Aqua bidest.

- : absence
+ : presence

First experiment

Table 5:

Number of revertant colonies per plate and mean values
using Escherichia coli strain WP2uvrA

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	34	37	31	34
4	-	36	47	31	31
20	-	40	39	36	45
100	-	39	37	35	44
500	-	42	46	39	41
2500	-	38	49	28	37
10000	-	32	36	30	31
0	+	42	38	55	34
4	+	37	38	41	33
20	+	44	45	43	43
100	+	40	44	38	38
500	+	51	71	49	33
2500	+	46	48	44	45
10000	+	39	33	39	46

Compound dissolved in 100 microliter Aqua bidest.

\- : absence
\+ : presence

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Second experiment

Table 7: Mutagenicity experiment with Dodigen 4022 with and without metabolic activation

TA 100

Number of revertant colonies per plate and mean values using Salmonella typhimurium strain TA 100

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate			Surviving fraction
0	-	104	96	124	91	1.0
4	-	123	95	159	116	1.0
20	-	125	116	124	136	1.0
100	-	132	117	155	123	0.9
500	-	125	109	122	145	1.0
2500	-	135	125	141	138	0.8
5000	-	132	125	121	151	0.8
0	+	110	118	115	98	1.0
4	+	116	116	117	115	0.8
20	+	120	122	128	110	0.9
100	+	135	112	138	154	0.9
500	+	127	113	127	142	1.0
2500	+	134	150	126	126	0.9
5000	+	133	140	123	137	1.1

Compound dissolved in 100 microliter Aqua bidest.

- : absence
+ : presence

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Second experiment

Table 8: Mutagenicity experiment with Dodigen 4022 with and without metabolic activation

TA 1535

Number of revertant colonies per plate and mean values using Salmonella typhimurium strain TA 1535

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	10	7	11	11
4	-	8	10	7	7
20	-	10	3	13	13
100	-	11	13	4	16
500	-	14	15	12	16
2500	-	12	10	11	14
5000	-	11	13	13	6
0	+	11	13	13	7
4	+	15	21	8	16
20	+	11	10	11	12
100	+	13	9	13	16
500	+	10	16	9	6
2500	+	11	11	11	12
5000	+	13	13	14	13

Compound dissolved in 100 microliter Aqua bidest.

- : absence
+ : presence

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Second experiment

Table 9: Mutagenicity experiment with Dodigen 4022 with and without metabolic activation

TA 1537

Number of revertant colonies per plate and mean values using Salmonella typhimurium strain TA 1537

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	6	4	5	8
4	-	6	3	7	7
20	-	5	5	5	5
100	-	7	6	5	9
500	-	9	14	4	8
2500	-	10	8	12	11
5000	-	9	6	11	11
0	+	8	6	5	12
4	+	6	7	4	7
20	+	7	6	6	8
100	+	10	8	11	12
500	+	9	12	8	6
2500	+	6	6	7	6
5000	+	9	4	9	13

Compound dissolved in 100 microliter Aqua bidest.

- : absence
+ : presence

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Second experiment

Table 10: Mutagenicity experiment with Dodigen 4022
with and without metabolic activation

TA 1538

Number of revertant colonies per plate and mean values
using Salmonella typhimurium strain TA 1538

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	16	17	15	15
20	-	16	22	11	14
100	-	16	10	18	19
500	-	16	14	15	18
2500	-	24	20	18	33
5000	-	24	19	22	31
10000	-	27	27	31	24
0	+	21	20	19	24
20	+	22	19	20	26
100	+	20	25	20	16
500	+	25	18	26	30
2500	+	29	32	26	28
5000	+	28	34	27	23
10000	+	27	25	28	28

Compound dissolved in 100 microliter Aqua bidest.

\- : absence
\+ : presence

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Second experiment

Table 11: Mutagenicity experiment with Dodigen 4022 with and without metabolic activation

TA 98

Number of revertant colonies per plate and mean values using Salmonella typhimurium strain TA 98

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	30	29	29	31
20	-	25	23	24	27
100	-	26	21	28	30
500	-	32	28	32	37
2500	-	32	37	33	26
5000	-	33	34	27	38
10000	-	43	41	50	39
0	+	37	40	29	41
20	+	34	28	34	39
100	+	28	34	20	29
500	+	47	44	52	45
2500	+	46	56	48	35
5000	+	54	50	55	57
10000	+	45	39	44	52

Compound dissolved in 100 microliter Aqua bidest.

\- : absence
\+ : presence

Second experiment

Table 12: Mutagenicity experiment with Dodigen 4022 with and without metabolic activation

WP2uvrA

Number of revertant colonies per plate and mean values using Escherichia coli strain WP2uvrA

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate		
0	-	27	28	30	24
4	-	26	25	28	26
20	-	24	25	21	26
100	-	31	34	30	28
500	-	34	34	34	33
2500	-	33	29	32	39
5000	-	31	33	30	29
0	+	35	28	43	33
4	+	31	29	27	36
20	+	32	25	40	32
100	+	34	32	30	40
500	+	33	35	36	29
2500	+	40	36	36	49
5000	+	35	23	41	40

Compound dissolved in 100 microliter Aqua bidest.

\- : absence
\+ : presence

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Third experiment

Table 13: Mutagenicity experiment with Dodigen 4022
with metabolic activation

TA 98

Number of revertant colonies per plate and mean values
using Salmonella typhimurium strain TA 98

Dose ug/plate	Metabolic activation	Mean value	Colonies per plate			
0	+	28	24	30	34	23
20	+	30	21	24	37	37
100	+	32	29	35	31	32
500	+	34	25	31	42	39
2500	+	31	26	26	34	36
5000	+	38	38	33	39	42
10000	+	49	49	44	54	48

Compound dissolved in 100 microliter Aqua bidest.

\+ : presence

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First experiment

Table 14 : mutability (positive controls) and sterility test of the experiment with Dodigen 4022

Number of revertant colonies per plate and mean values using Salmonella typhimurium strains and Escherichia coli

Strain	Compound	Dose ug/plate	metab. Activ.	Mean value	Colonies per plate		
TA100	Sodium-azide	1	-	492	508	490	478
TA1535	Sodium-azide	1	-	332	330	351	315
TA1537	9-Aminoacridine	50	-	69	85	61	60
TA1538	2-Nitrofluorene	2.5	-	662	731	613	642
TA98	2-Nitrofluorene	2.5	-	481	505	465	472
WP2uvrA	MNNG	2.5	-	229	226	215	245
-	Dodigen 4022	10000	-	0	0	0	0

- : absence

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First experiment

Table 14a : mutability (positive controls) and sterility test of the experiment with Dodigen 4022

Number of revertant colonies per plate and mean values using Salmonella typhimurium strains and Escherichia coli

Strain	Compound	Dose ug/plate	metab. Activ.	Mean value	Colonies per plate		
TA100	2-Aminoanthracen	0.5	+	1559	1514	1591	1571
TA1535	2-Aminoanthracen	1	+	113	105	123	110
TA1537	2-Aminoanthracen	1	+	200	210	206	183
TA1538	2-Aminoanthracen	0.5	+	1138	1086	1200	1129
TA98	2-Aminoanthracen	0.5	+	1415	1304	1394	1546
WP2uvrA	2-Aminoanthracen	10	+	261	271	239	274
-	S-9 mix	500 ul	+	0	0	0	0
-	Dodigen 4022	10000 ug	+	0	0	0	0

+ : presence

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Second experiment

Table 15 : mutability (positive controls) and sterility test of the experiment with Dodigen 4022

Number of revertant colonies per plate and mean values using Salmonella typhimurium strains and Escherichia coli

Strain	Compound	Dose ug/plate	metab. Activ.	Mean value	Colonies per plate		
TA100	Sodium-azide	1	-	324	307	353	311
TA1535	Sodium-azide	1	-	402	394	419	392
TA1537	9-Aminoacridine	50	-	156	113	151	203
TA1538	2-Nitrofluorene	2.5	-	763	757	785	747
TA98	2-Nitrofluorene	2.5	-	588	607	592	566
WP2uvrA	MNNG	2.5	-	233	185	243	272
-	Dodigen 4022	5000	-	0	0	0	0

: absence

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Second and third experiment

Table 15a : mutability (positive controls) and sterility test of the experiment with Dodigen 4022

Number of revertant colonies per plate and mean values using Salmonella typhimurium strains and Escherichia coli

Strain	Compound	Dose ug/plate	metab. Activ.	Mean value	Colonies per plate		
TA100	2-Aminoanthracen	0.5	+	833	783	860	855
TA1535	2-Aminoanthracen	1	+	91	96	80	98
TA1537	2-Aminoanthracen	1	+	121	137	120	106
TA1538	2-Aminoanthracen	0.5	+	1014	1183	967	893
TA98	2-Aminoanthracen	0.5	+	1552	1561	1572	1522
WP2uvrA	2-Aminoanthracen	10	+	201	204	205	195
TA98*	2-Aminoanthracen	0.5	+	1347	1394	1285	1361
-	S-9 mix	500 ul	+	0	0	0	0
-	Dodigen 4022	5000 ug	+	0	0	0	0

\+ : presence
* : third experiment

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7. REFERENCES AND GUIDELINES

A. REFERENCES

1) B.N. Ames, W.W. Durston, E. Yamasaki and F.D. Lee, Carcinogens are mutagens. A simple test system combining liver homogenate for activation and bacteria for detection, Proc. Nat. Acad. Sci. USA 70 (1973) 2281 - 2285.

2) B.N. Ames, J. McCann and E. Yamasaki: Methods for detecting carcinogens and mutagens with the Salmonella / mammalian-microsome mutagenicity test, Mutation Res. 31 (1975) 347 -364.

3) M.H.L. Green amd W.J. Muriel: Mutagen testing using trp^+ reversion in Escherichia coli, Mutation Res. 38 (1976) 3 - 32.

4) A.P. Alvares, D.R. Bickers and A. Kappas: Polychlorinated biphenyls: a new type of inducer of cytochrome P 448 in the liver. Proc. Nat. Acad. Sci. USA 70 (1973) 1321 - 1325.

5) J. McCann, N.E. Springarn, J. Kobory and B.N. Ames: Detection of carcinogens as mutagens: bacterial tester strains with R factor plasmids, Proc. Nat. Acad. Sci. USA 72 (1975) 979 - 983.

B. GUIDELINES

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

- OECD Guideline for testing of chemicals 471
  Genetic Toxicology : Salmonella typhimurium, Reverse Mutation Assay,
  Adopted : May 26th, 1983

- OECD Guideline for testing of chemicals 472
  Genetic Toxicology : Escherichia coli, Reverse Mutation Assay,
  Adopted : May 26th, 1983

- The Salmonella typhimurium reverse mutation assay
  HG-Gene Muta-S. typhimurium, August 1982
- The Escherichia coli WP2 and WP2 uvrA reverse mutation assay
  HG-Gene Muta-E. coli, August 1982
  Office of Toxic Substances
  Office of Pesticides and Toxic Substances
  U.S. Environmental Protection Agency
  Washington, D.C. 20460

- EEC Directive 79/831 Annex V, 4.3.1

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04701

[redacted]

GB-E, ETH-I
D 562

Produkt: Hoe S 4022/ Dodigen 4022
Chargen-Nr.: E 06159865
Hoe.Nr.: HOE CG 0351 OE ZD82 0001

Analytisches Laboratorium: ~~XXXXXXX~~ CG 0
~~XXXXXXXXXXXXXXXXXX~~

Datum: 20.08.91

Untersuchungsbefund

w(HOE CG 0351 OE ZD82 0001)= 82.0 % (w/w))

Nebenkomponenten und Anmerkungen siehe Anlage
Rückstellmuster werden in den Archiven des Analytischen Labors gelagert

Die Lagerstabilität beträgt bei 20°C ca. 0,5 Jahre,
bei 0°C ca. 2 Jahre.

Leiter des
ANALYTISCHEN LABORATORIUMS

[redacted]

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