Annex	II	Genotoxicity testing - In vitro studies
Point addressed	5.8.1	

1.2	Title	Salmonella and Escherichia/mammalian-microsome mutagenicity test
1.3	Report and/or project N° Ciba File N° (Desire)	963102 62826 / 10
1.4	Lab. Report N°	963102
1.5	Cross reference to original study / report	5.8.1/06
1.6	Authors	Report: Summary:
1.7	Date of report	January 10, 1997
1.8	Published / owner	No, Novartis Crop Protection AG
2.1	Testing facility	Toxicology/Genetic Toxicology, Novartis Crop Protection AG, Basel, Switzerland
2.2	Dates of experimental work	September 18, 1996 to January 10, 1997
3.	Objectives	To detect a mutagenic activity at the level of gene mutations in bacteria.
4.1	Test substance	CGA 62826 tech. (Metabolite of CGA 48988 and CGA 329351)
4.2	Specification	Batch no. RV-1592/4 / purity 100%
4.3	Storage stability	Reanalysis date: August 31, 1999
4.4	Stability in vehicle	Stable under the conditions of the test (see analytical results §13)
4.5	Homogeneity in vehicle	Not applicable (solution)
4.6	Validity	Not applicable
5	Vehicle / solvent	Dimethylsulfoxide
6	Physical form	Solid
7.1	Test method	Directive 92/69/EEC Method B. 14 (1992), OECD 471 (1983), US-EPA health effects testing guideline §798.5265 (1987), Japan MHW (1986)
7.2	Justification	Required by regulatory authorities
7.3	Copy of method	Not applicable
8	Choice of method	Required by regulatory authorities
9	Deviations from EEC Directive 92/69 B. 14	No statistical test was run on the study results.
10.1	Certified laboratory	Yes
10.2	Certifying authority	Swiss Federal Department of the Interior
10.3	GLP	Yes
10.4	Justification	Not applicable
11.1	GEP	Not applicable

11.2 Type of facility (official or officially recognised) Not applicable

11.3 Justification Not applicable

12 Test system

Bacterial strains:

strain		type of mutation
Salmonella typhimurium	TA 100	base-pair substitution
Salmonella typhimurium	TA 1535	base-pair substitution
Escherichia coli	WP2 uvrA	base-pair substitution
Salmonella typhimurium	TA 102	base-pair substitution
Salmonella typhimurium	TA 98	frame-shift
Salmonella typhimurium	TA 1537	frame-shift

Metabolic activation:

Post mitochondrial supernatant (S9 fraction) from Tif RAIf(SPF) Sprague-Dawley derived adult male rats. S9 fraction was prepared by the performing laboratory. For enzyme induction the animals received a single injection (i.p.) of Aroclor 1254 (500 mg/kg b.w.) 5 days prior to sacrifice.

Doses:

Toxicity test:	20.58	-5000.00	µg/plate
original mutagenicity test:	312.50	-5000.00	µg/plate
confirmatory mutagenicity test:	312.50	-5000.00	µg/plate

Negative control (solvent):

Positive controls (with S9):

strain	compound	concentration
TA 100	2-aminoanthracene	1.5 µg/plate
TA 1535	cyclophosphamide	200.0 µg/plate
WP2 uvrA	2-aminoanthracene	20.0 µg/plate
TA 102	2-aminoanthracene	5.0 µg/plate*
TA 98	2-aminoanthracene	1.5 µg/plate
TA 1537	2-aminoanthracene	1.5 µg/plate

* Preincubation assay:

TA 102	2-aminoanthracene	4.0 µg/plate

Positive controls (without S9):

strain	compound	concentration
TA 100	sodium azide	2.0 µg/plate
TA 1535	sodium azide	2.0 µg/plate
WP2 uvrA	4-nitroquinoline	2.0 µg/plate
TA 102	Mitomycin-C	0.5 µg/plate
TA 98	2-nitrofluorene	5.0 µg/plate
TA 1537	9-aminoacridine	80.0 µg/plate

Application:

Both mutagenicity tests without metabolic activation and the first experiment with metabolic activation were conducted as standard plate incorporation assay. The second experiment with metabolic activation was conducted as preincubation assay.
0.1 ml of the overnight cultures were mixed with top agar, sodium phosphate buffer or activation mixture and 0.1 ml of a solution of the test substance, the positive control or the solvent as a negative control and poured on minimal agar plates. In the experiment with Salmonella the top agar was supplemented with traces of L-histidine and d-biotin. In the experiment with E. coli it was supplemented with traces of L-tryptophan. In the preincubation assay, bacteria were incubated with the test substance and the activation mixture for 30 min at 37°C before the addition of top agar. Plates were incubated for about 48 h at 37°C. The *toxicity test* was run with strains TA 100 and WP2 uvrA using one plate per concentration and control. The *mutagenicity tests* were run with all six strains and three plates per concentration and control.

Measurements:

From each plate the number of revertant colonies (mutants) was determined. Means and mutant factors were calculated. Observations such as precipitates or reduced background lawn were recorded.

Evaluation criteria:

The test substance will be considered positive in the test system if one or both of the following conditions are met:
At least a reproducible doubling of the mean revertants per plate above that of the negative control at any concentration for one or more of strains TA 98, TA 1535, TA 1537 or WP2 uvrA.
A reproducible increase of the mean revertants per plate at any concentration above that of the negative control by at least a factor of 1.5 for strains TA 100 or TA 102.
Generally a concentration-related effect should be demonstrable.

13 Findings

Analytical results:

The concentrations of the two samples analysed were found to be 97.7 and 93.1% of the respective nominal concentrations.

Toxicity test:

5000.00 µg/ml was found to be the highest suitable concentration for the original mutagenicity experiment with and without metabolic activation.

Mutagenicity tests: (see table below)

In both experiments performed, with and without metabolic activation, no significant increase in the incidence of either histidine- or tryptophan-prototrophic mutants was observed at any concentration in comparison with the negative control. By contrast, the positive controls resulted in clearly increased values in both experiments.

Observations:

No precipitates or aggregates of the test material were observed on the agar plates. Normal background growth was observed with all strains at all concentrations.

Summary of the results of the original mutagenicity experiment with metabolic activation (mean revertants per plate)

Treatment		TA 100	TA 1535	WP2 uvrA	TA 102	TA 98	TA 1537
negative control		128.3	11.3	20.7	295.7	21.3	10.0
positive control		2343.7	247.3	1067.0	2180.0	1202.7	197.3
312.50	µg/plate	125.7	15.3	20.7	319.7	24.3	8.7
625.00	µg/plate	138.3	12.0	20.3	288.7	22.0	10.3
1250.00	µg/plate	151.7	9.0	18.0	309.0	23.3	8.0
2500.00	µg/plate	143.7	14.0	22.3	277.0	24.3	7.7
5000.00	µg/plate	144.0	13.7	15.7	262.7	24.7	7.7

Summary of the results of the original mutagenicity experiment without metabolic activation (mean revertants per plate)

Treatment		TA 100	TA 1535	WP2 uvrA	TA 102	TA 98	TA 1537
negative control		139.0	11.3	20.0	274.7	16.3	8.3
positive control		1168.0	861.7	488.7	1245.7	522.0	1114.3
312.50	µg/plate	135.0	13.3	18.3	291.7	20.0	7.3
625.00	µg/plate	133.0	11.3	18.0	276.3	15.3	9.7
1250.00	µg/plate	106.7	17.7	18.0	274.7	17.3	8.0
2500.00	µg/plate	132.7	17.7	18.7	235.3	15.7	10.0
5000.00	µg/plate	125.3	11.0	14.0	238.0	21.0	4.0

Summary of the results of the confirmatory mutagenicity experiment with metabolic activation (mean revertants per plate)

Treatment		TA 100	TA 1535	WP2 uvrA	TA 102	TA 98	TA 1537
negative control		137.0	11.7	26.7	284.7	43.7	12.7
positive control		660.3	353.7	258.3	1107.0	625.3	122.3
312.50	µg/plate	120.3	16.3	26.3	272.7	42.0	11.7
625.00	µg/plate	124.7	17.7	30.0	257.7	44.3	12.0
1250.00	µg/plate	131.0	15.7	25.0	249.7	42.7	13.3
2500.00	µg/plate	129.7	14.3	25.7	244.0	41.3	13.7
5000.00	µg/plate	122.0	14.7	19.3	195.7	41.7	14.3

Summary of the results of the confirmatory mutagenicity experiment without metabolic activation (mean revertants per plate)

Treatment		TA 100	TA 1535	WP2 uvrA	TA 102	TA 98	TA 1537
negative control		134.0	19.3	25.3	258.0	26.7	9.3
positive control		1180.3	771.3	482.0	1234.7	413.7	924.3
312.50	µg/plate	119.7	19.3	24.7	231.3	27.3	10.3
625.00	µg/plate	127.7	15.0	21.7	235.0	24.0	8.3
1250.00	µg/plate	121.7	14.3	19.7	173.0	26.0	8.0
2500.00	µg/plate	115.3	15.3	22.7	135.3	28.0	8.3
5000.00	µg/plate	102.3	13.0	22.3	22.3	30.3	6.7

Conclusion:

No evidence for a mutagenic effect was found in this in vitro test system.

14	**Statistics**	None
15	**References (published)**	None
16	**Unpublished data**	None

CP 2.322/E.D./August 05, 1997

10

SALMONELLA AND ESCHERICHIA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

Test Number: 963102
Test Substance: CGA 62826 tech. (metabolite of CGA 48988)

FINAL REPORT

Author: Mr. 5.1.2e Woo

Testing Facility: Genetic Toxicology
Novartis Crop Protection Inc.
(successor in business of Sandoz Ltd. and Ciba-Geigy Ltd.)
Basle, Switzerland

Test Guidelines: OECD 471 (1983)
EPA § 798.5265 (1987)
EEC B.14 (1992)
MITI (1986)

Final Report issued: January 10, 1997

Sponsor: Novartis Crop Protection Inc.
(successor in business of Sandoz Ltd. and Ciba-Geigy Ltd.)
Basel, Switzerland

Volume 1 of 1 of submission

This report contains 64 numbered pages plus 11 appended pages

- Appendix 1: Personal Records of Staff
- Appendix 2: Report of Analytical Determinations

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CERTIFICATION OF GOOD LABORATORY PRACTICE

This study was performed in compliance with Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986 [Verfahren und Grundsätze der Guten Laborpraxis (GLP) in der Schweiz, März 1986], issued by the Federal Department of the Interior and the Intercantonal Office for the Control of Medicaments, Switzerland. These procedures are in essence consistent with:

- OECD Principles of Good Laboratory Practice (Council Decision 81/30, adopted on May 12, 1981, and the OECD Recommendation 89/87 concerning the 'Compliance with Principles of Good Laboratory Practice', adopted on October 2, 1989).
- United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 160 (FIFRA); Federal Register, August 17, 1989.
- United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 792 (TSCA); Federal Register, August 17, 1989.
- Japan Ministry of Agriculture, Forestry and Fisheries (MAFF), NohSan, Notification No. 3850, Agricultural Production Bureau, August 10, 1984.

Study Director: Mr. [redacted]

Date: January 10, 1997

Facility Management: Dr. [redacted]

Date: January 10, 1997

For the Sponsor:

[redacted]

Date: January 31, 1997

PAGE RESERVED FOR FLAGGING STATEMENTS

SIGNATURES

This report represents the results of the investigations as compiled by the undersigned.

Study Director: Mr. 5.1.2.e Woo

Date: January 10, 1997

Facility Management: Dr. 5.1.2.e Woo

Date: January 10, 1997

Quality Assurance Statement

Novartis Crop Protection Inc., GLP Quality Assurance Product Safety, 4002 Basel
(Successor in business of Ciba-Geigy Ltd. and Sandoz Ltd.)

Project	963102
Test Substance	CGA 62826 tech.
Study Title	Salmonella and Escherichia/Mammalian-Microsome Mutagenicity Test
Study Director	5.1.2.e Woo
QA-Inspector	

I hereby certify that the following Quality Assurance activities were performed:

Activity	Performed	Reported
Facility Inspection	March 06, 1996	March 11, 1996
Facility Inspection	March 28, 1996	April 01, 1996
Process Based Inspection	July 02, 1996	July 02, 1996
Protocol Audit	September 06, 1996	September 06, 1996
Facility Inspection	September 19, 1996	September 20, 1996
Final Report Audit	December 05, 1996	December 05, 1996

January 30, 1997

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Date
Form. QSSTAT12

5.1.2.e Woo

.....
5.1.2.e Woo, subst.
Inspector Quality Assurance

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COMPOUND INFORMATION

Test material:	CGA 62826 tech. (metabolite of CGA 48988)
Batch No.:	RV-1592/4
Purity:	100%
Stability (of the test compound itself):	Stable (see reanalysis date)
Stability (in the vehicle used, under the conditions of the test):	Stable
Appearance:	Solid
Reanalysis date:	August 31, 1999
Storage conditions:	<+10°C
Material submitted by:	Novartis Crop Protection Inc. Basle, Switzerland

GENERAL

Type of study: Salmonella and Escherichia/Mammalian-Microsome Mutagenicity Test. According to SOP No. 30 50 03, Novartis Crop Protection Inc., Basle, Switzerland.

The test procedure is based on:

- OECD guideline [1]
- EEC guideline [2]
- MITI guideline [3]
- EPA guideline [4]

Purpose: To evaluate the test compound for mutagenic activity in bacterial test systems in the presence and absence of a rat liver metabolic activation system

Test organisms: Strains of Salmonella typhimurium:
TA 98, TA 100, TA 102, TA 1535, TA 1537

Strain of Escherichia coli: E. coli WP2 uvrA

Testing facility: Novartis Crop Protection Inc.
Basle, Switzerland
Genetic Toxicology

Archives: Archives of Genetic Toxicology
Novartis Crop Protection Inc.
Basle, Switzerland

Personnel: Technical conduct:

Mrs. [redacted]
Mrs. [redacted]

Study Director: Mr. [redacted]

Principal Investigator:
(responsible for analytical study) Mr. [redacted]

Novartis Crop Protection Inc.
Basle, Switzerland
Laboratories of Cell Biology

Sponsor:	Novartis Crop Protection Inc. Basle, Switzerland
Sponsor monitoring scientist:	Dr. [redacted]
Test number:	963102
Vehicle:	DMSO
Concentration range in the range finding test:	20.6 to 5000.0 µg/plate
Concentration ranges in the mutagenicity tests:	*Original experiment* 312.5 to 5000.0 µg/plate *Confirmatory experiment* 312.5 to 5000.0 µg/plate
Study initiation date:	September 04, 1996
Experimental start date:	September 18, 1996
Experimental termination date:	October 17, 1996
Study termination date:	January 10, 1997

The company Novartis Crop Protection Inc. has resulted from the merger of the companies Ciba-Geigy Ltd. and Sandoz Ltd. and is partial successor in business from above-mentioned companies. This applies also to all aspects concerned with the requirements of Good Laboratory Practice.

ABSTRACT

CGA 62826 tech. (metabolite of CGA 48988), identified as solid, purity 100%, batch no. RV-1592/4, was tested for mutagenic effects in vitro in histidine-requiring strains of Salmonella typhimurium and in a tryptophan-requiring strain of Escherichia coli. The following strains were used: S. typhimurium TA 98, TA 100, TA 102, TA 1535, TA 1537 and E. coli WP2 uvrA. The test was performed with and without the addition of rat-liver post mitochondrial supernatant (S9 fraction) as an extrinsic metabolic activation system. The compound was dissolved in DMSO and tested at five concentrations in the range of 312.5 to 5000.0 µg/plate in the presence and absence of a metabolic activation system. In order to confirm the results, the experiments were repeated with and without metabolic activation at five concentrations in the range of 312.5 to 5000.0 µg/plate. Each strain was additionally tested in the presence and in the absence of a metabolic activation system with a suitable, known mutagen as positive control.

The original experiment with and without metabolic activation and the confirmatory experiment without activation were performed as standard plate incorporation assay. The confirmatory experiments with metabolic activation were carried out as preincubation assay.

In both experiments, performed with and without metabolic activation, none of the tested concentrations of CGA 62826 tech. led to an increase in the incidence of either histidine- or tryptophan-prototrophic mutants by comparison with the negative control.

CONCLUSION

Based on the results of these experiments and on standard evaluation criteria, it is concluded that CGA 62826 tech. (metabolite of CGA 48988) and its metabolites did not induce gene mutations in the strains of S. typhimurium and E. coli used.

RATIONALE

This test permits the detection of gene mutations induced by the test material or its metabolites in histidine-requiring strains of Salmonella typhimurium and in a tryptophan-requiring strain of Escherichia coli [5-11].

When the Salmonella strains are exposed to a mutagen, some of the bacteria in the treated population, through chemical interaction with the compound or its metabolites, undergo genetic changes which cause them to revert to a non-histidine-requiring state and thus to grow in the absence of exogenous histidine . Similarly, after mutation, the Escherichia coli bacteria are able to grow in tryptophan-free medium. Mutagenic effects of the test substance are demonstrable on comparison of the number of bacteria in the treated and control cultures that have undergone reverse-mutation to histidine prototrophism or tryptophan prototrophism, respectively. Different tester strains are used because of differing sensitivities to known mutagens. The following bacterial strains have been used in this study:

Strain	Type of Mutation
S. typhimurium TA 100	base-pair substitution
S. typhimurium TA 1535	base-pair substitution
E. coli WP2 uvrA	base-pair substitution
S. typhimurium TA 102	base-pair substitution
S. typhimurium TA 98	frame-shift
S. typhimurium TA 1537	frame-shift

PROCEDURE

Source of strains

The histidine-auxotrophic strains of Salmonella typhimurium (TA 98, TA 102, TA 1535) were obtained from Prof. 5.1.2.e Woo Berkeley, USA (1983). Strain TA 100 was obtained from Dr. 5.1.2.e 5.1.2.e Woo Hoffmann-La Roche Limited, Basel, Switzerland (1986). Strain TA 1537 was obtained from Dr. 5.1.2.e Woo Hoffmann-La Roche Limited, Basel, Switzerland (1994). The tryptophan-auxotrophic strain of Escherichia coli (WP2 uvrA) was obtained from the National Collection of Industrial Bacteria, Aberdeen, Scotland (1977).

Source of chemicals

Chemical	Quality	Purity	Supplier
2-Aminoanthracene	practicum	*	Sigma, USA
2-Nitrofluorene	p.a.	>98.0%	Merck, Germany
4-Nitroquinoline	purum	>97.0%	Fluka, Switzerland
9-Aminoacridine	purum	98.5%	Fluka, Switzerland
Aroclor 1254	*	*	Analabs, USA
Cyclophosphamide	*	97.0%	NBS Biologicals, England
Sodium azide	purum	>99.0%	Fluka, Switzerland
Mitomycin-C	*	*	Syntex Pharm AG, Switzerland
DMSO	p.a.	99.5%	Merck. Germany

* No data available

Preparation of the bacterial cultures

Aliquots from frozen stocks were grown in liquid nutrient broth medium (NB-medium) for 8 hours and then used for the experiment.

Control of the genotype of the strains

The characteristics of the strains were checked every two months. Histidine-auxotrophy of the Salmonella strains was demonstrated by the requirement for L-histidine. The presence of the rfa character was assayed by the sensitivity for crystal-violet. The deletion of the uvrB gene was demonstrated by the sensitivity for UV-light. The Salmonella strains containing the R-factor (TA 98 and TA 100) were additionally checked for ampicillin resistance. The tryptophan-auxotrophy of E. coli WP2 uvrA was demonstrated by the requirement for tryptophan. The absence of the uvrA gene was demonstrated by the sensitivity of the strain for UV-light. Furthermore, all strains were checked for their characteristic reversion properties with known mutagens (positive controls).

Preparation of the metabolic activation mixture

Rat-liver post mitochondrial supernatant (S9 fraction) was prepared in advance from male RAI rats (Tif: RAIf [SPF]), reared at the Animal Farm of CIBA-GEIGY Limited, Sisseln, Switzerland. The animals were treated with Aroclor 1254 (500 mg/kg, i.p.) 5 days prior to sacrifice. The livers were homogenized with 3 volumes of 150 mM KCl. The homogenate was centrifuged for 15 minutes at 9000x g and the resulting supernatant (S9 fraction) was stored at approximately -80°C for no longer than one year. The protein content of the S9 fraction was 30.31 mg/ml.

The activation mixture contained:

Rat liver S9 fraction	100.0 µl/ml
NADP	4.0 µmol/ml
$MgCl_2$	8.0 µmol/ml
KCl	33.0 µmol/ml
Na-phosphate-buffer, pH 7.4	100.0 µmol/ml
Glucose-6-phosphate	5.0 µmol/ml

Solubilisation of the test substance

CGA 62826 tech. (metabolite of CGA 48988) was dissolved in DMSO at room temperature. The test substance CGA 62826 tech. (metabolite of CGA 48988) was soluble up to the concentration of 50 mg/ml. Lower concentrations of the test substance were obtained by appropriate dilution of the stock solution with DMSO. No precipitates or aggregates were noted.

Analytical control

To demonstrate that the test system was exposed to the intended concentrations of the test substance in the mutagenicity tests, the concentration of the substance in solution has been determined by the analytical unit. The analysis was performed with the lowest concentration, which was obtained by serial dilution of the highest concentration used.

Setting up of the test plates

Standard plate incorporation assay: 0.1 ml of the overnight cultures were mixed with 2 ml of top agar, either 0.5 ml of 100 mM sodium phosphate buffer (experiments without activation) or 0.5 ml of the activation mixture (experiments with activation) and 0.1 ml of a solution of the test substance, the positive control or the solvent as a negative control and poured on minimal agar in Petri dishes.

Preincubation assay: 0.1 ml of the overnight cultures were mixed with 0.5 ml of the activation mixture and 0.1 ml of a solution oft the test substance, the positive control or the solvent as a negative control and incubated for 30 min. at 37°C. Thereafter 2 ml of top agar were added to the mixtures and they were poured on minimal agar in Petri dishes.

Each Petri dish contained about 20.0 ml of minimal agar (1.5% agar supplemented with 2% salts of the Vogel-Bonner Medium E and 2% glucose). The top agar was composed of 0.6% agar and 0.6% NaCl. In the experiment with Salmonella the top agar was supplemented with 10% of 0.5 mM L-histidine and 0.5 mM d-biotin dissolved in water. In the experiment with E. coli it was supplemented with 10% of 0.5 mM L-tryptophan dissolved in water.

Preliminary range finding test

A range finding test was carried out with strains S. typhimurium TA 100 and E. coli WP2 uvrA with and without metabolic activation at six concentrations of the test substance and one negative control according to a Standard Operating Procedure of Genetic Toxicology. The highest concentration applied was 5000.0 µg/plate. The five lower concentrations decreased by a factor of three. The plates were inverted and incubated for about 48 hours at 37±1.5°C in darkness. Thereafter, they were evaluated by counting the colonies and determining the background lawn. One plate per test substance concentration and negative control was used.

Mutagenicity test

The mutagenicity test was performed with the Salmonella typhimurium strains TA 98, TA 100, TA 102, TA 1535, TA 1537 and with the Escherichia coli strain WP2 uvrA with and without metabolic activation according to Standard Operating Procedures of Genetic Toxicology. Each of the five concentrations of the test substance, a negative and a positive control were tested, using three plates per test substance concentration and controls. The highest concentration applied was determined in the preliminary range finding test and the four lower concentrations decreased by a factor of two. The plates were inverted and incubated for about 48 hours at 37±1.5°C in darkness. Thereafter, they were evaluated by counting the number of colonies and determining the background lawn.

Negative and positive controls

The solvent alone was used as the negative control. The positive controls were the following reference mutagens:

Experiment with metabolic activation			
Strain	**Mutagen**	**Solvent**	**Concentration**
TA 100	2-Aminoanthracene	DMSO	1.5 µg/plate
TA 1535	Cyclophosphamide	Bidistilled water	200.0 µg/plate
WP2 uvrA	2-Aminoanthracene	DMSO	20.0 µg/plate
TA 102	2-Aminoanthracene	DMSO	5.0 µg/plate *
TA 98	2-Aminoanthracene	DMSO	1.5 µg/plate
TA 1537	2-Aminoanthracene	DMSO	1.5 µg/plate

* 4.0 µg/plate in the preincubation assay

Experiment without metabolic activation			
Strain	**Mutagen**	**Solvent**	**Concentration**
TA 100	Sodium azide	Bidistilled water	2.0 µg/plate
TA 1535	Sodium azide	Bidistilled water	2.0 µg/plate
WP2 uvrA	4-Nitroquinoline (4-NQO)	DMSO	2.0 µg/plate
TA 102	Mitomycin-C	Bidistilled water	0.5 µg/plate
TA 98	2-Nitrofluorene	DMSO	5.0 µg/plate
TA 1537	9-Aminoacridine	DMSO	80.0 µg/plate

Colony counting and scoring of the plates

Colonies were counted electronically using an Artek Colony Counter (Fisher Scientific), or manually where minor agar damage or test chemical precipitates or strong coloration of the agar plates might have interfered with automating counting. The results were sent on line to a computer. They were checked on a random basis by the operator. Observations indicating precipitates of the test substance in the top agar or a reduced or absent bacterial background lawn were registered additionally. Means for all mutagenicity assays were calculated and included in the Results section.

Assay acceptance criteria

A test is considered acceptable if the mean colony counts of the negative control values of all strains are within the acceptable ranges and if the results of the positive controls meet the criteria for a positive response. In either case the final decision is based on the scientific judgement of the Study Director.

Criteria for a positive response

The test substance will be considered to be positive in the test system if one or both of the following conditions are met:

- At least a reproducible doubling of the mean number of revertants per plate above that of the negative control at any concentration for one or more of the following strains: TA 98, TA 1535, TA 1537, E. coli WP2 uvrA.
- A reproducible increase of the mean number of revertants per plate for any concentration above that of the negative control by at least a factor of 1.5 for strains TA 100 or TA 102.

Generally a concentration-related effect should be demonstrable.

Statistics

A statistical analysis was not performed. At present the use of statistical methods concerning this particular test system is not generally recommended [8].

Negative and positive historical control data and acceptable ranges for negative controls

Arithmetic Mean and Standard Deviation (SD) of colony counts reported in N separate experiments over the period of January 01, 1995 to December 31, 1995 and acceptable ranges for mean colony counts of spontaneous revertants.

With metabolic activation, standard plate incorporation assay						
Strain	Substance	µg/plate	Mean	SD	N	Acceptable range
TA 100	Negative control		139.28	27.85	152	70-220
	2-Aminoanthracene	1.5	1807.39	359.14	152	
TA 1535	Negative control		15.09	3.00	152	7-35
	Cyclophosphamide	200.0	266.58	81.04	152	
WP2 uvrA	Negative control		28.46	7.02	70	8-50
	2-Aminoanthracene	20.0	907.25	222.54	70	
TA102	Negative control		286.59	44.99	72	150-360
	2-Aminoanthracene	5.0	1560.29	359.70	72	
TA 98	Negative control		35.82	7.70	152	20-70
	2-Aminoanthracene	1.5	1807.78	348.13	152	
TA 1537	Negative control		13.29	3.73	152	5-30
	2-Aminoanthracene	1.5	274.47	85.44	152	

With metabolic activation, preincubation assay						
Strain	Substance	µg/plate	Mean	SD	N	Acceptable range
TA 100	Negative control		135.85	32.23	18	70-220
	2-Aminoanthracene	1.5	1738.86	422.61	18	
TA 1535	Negative control		15.70	4.30	18	7-35
	Cyclophosphamide	200.0	300.63	59.64	18	
WP2 uvrA	Negative control		nd	nd	0	8-50
	2-Aminoanthracene	20.0	nd	nd	0	
TA102	Negative control		nd	nd	0	150-360
	2-Aminoanthracene	5.0	nd	nd	0	
TA 98	Negative control		40.20	8.88	18	20-70
	2-Aminoanthracene	1.5	1519.74	496.10	18	
TA 1537	Negative control		14.72	4.62	18	5-30
	2-Aminoanthracene	1.5	241.63	91.50	18	

nd no data yet available

Without metabolic activation, standard plate incorporation assay						
Strain	Substance	µg/plate	Mean	SD	N	Acceptable range
TA 100	Negative control		122.65	31.37	170	70-220
	Sodium azide	2.0	1195.70	209.05	170	
TA 1535	Negative control		13.67	3.49	170	7-30
	Sodium azide	2.0	987.12	149.79	170	
WP2 uvrA	Negative control		20.14	5.05	70	8-40
	4-Nitroquinoline	2.0	738.20	142.71	70	
TA 102	Negative control		248.56	53.29	72	150-360
	Mitomycin-C	0.5	1379.99	297.58	72	
TA 98	Negative control		20.30	6.00	170	12-50
	2-Nitrofluorene	5.0	1722.79	306.27	170	
TA 1537	Negative control		9.32	3.22	170	3-20
	9-Aminoacridine	80.0	2085.55	509.69	170	

RESULTS

Range finding test

Six concentrations of CGA 62826 tech. ranging from 20.6 to 5000.0 µg/plate were tested with strain Salmonella typhimurium TA 100 and strain Escherichia coli WP2 uvrA to determine the highest concentration to be used in the mutagenicity assay. The experiments were performed with and without metabolic activation. Normal background growth was observed with both strains. The numbers of revertant colonies were not reduced. From the results obtained (Tables 1-4), the highest concentration suitable for the mutagenicity test was selected to be 5000.0 µg/plate with and without metabolic activation.

Mutagenicity test

In the **original experiments** performed with and without metabolic activation, treatment of strains TA 98, TA 100, TA 102, TA 1535, TA 1537 and WP2 uvrA with CGA 62826 tech. did not lead to an increase in the incidence of either histidine- or tryptophan-prototrophic mutants in comparison with the negative control (Tables 5, 6 and 9-20).

In the **confirmatory experiments** performed with and without metabolic activation, again after treatment of strains TA 98, TA 100, TA 102, TA 1535, TA 1537 and WP2 uvrA with CGA 62826 tech.) no increase in the incidence of either histidine- or tryptophan-prototrophic mutants was observed in comparison with the negative control (Tables 7, 8 and 21-32).

In the mutagenicity tests normal background growth was observed with all strains at all concentrations. The numbers of revertant colonies were not reduced with increasing concentration. Therefore, the test substance exerted no toxic effect on the growth of the bacteria.

Analytical control

To confirm that the cells were actually exposed to the intended test concentrations and to confirm the stability of the test substance in the vehicle used, determination of the concentration of the test substance in solution was performed by HPLC. The values found by analysis of the different samples were in agreement with the intended concentrations (97.7%* and 93.1%*), thus demonstrating a sufficient stability of the test substance in the vehicle.

There were no known circumstances or occurrences in this study that were considered to have affected the quality or integrity of the test data.

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* mean value of two independent determinations.

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LEGEND TO TABLES 1 TO 32

P	Precipitates
R	Background reduced
I	Background invisible
E	Error
M	Manual counting
-	No remarks

TABLE 1 : RANGE FINDING TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 100

Treatment	Colony counts	Mean
Negative control	141	
CGA 62826 tech.:		
20.58 µg/plate	115	
61.73 µg/plate	127	
185.19 µg/plate	136	
555.56 µg/plate	137	
1666.67 µg/plate	141	
5000.00 µg/plate	147	

Treatment	Remarks	Factor
Negative control	-	1.00
CGA 62826 tech.:		
20.58 µg/plate	-	0.82
61.73 µg/plate	-	0.90
185.19 µg/plate	-	0.96
555.56 µg/plate	-	0.97
1666.67 µg/plate	-	1.00
5000.00 µg/plate	-	1.04

TABLE 2 : RANGE FINDING TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 100

Treatment	Colony counts	Mean
Negative control	112	
CGA 62826 tech.:		
20.58 µg/plate	132	
61.73 µg/plate	141	
185.19 µg/plate	138	
555.56 µg/plate	151	
1666.67 µg/plate	132	
5000.00 µg/plate	128	

Treatment	Remarks	Factor
Negative control	-	1.00
CGA 62826 tech.:		
20.58 µg/plate	-	1.18
61.73 µg/plate	-	1.26
185.19 µg/plate	-	1.23
555.56 µg/plate	-	1.35
1666.67 µg/plate	-	1.18
5000.00 µg/plate	-	1.14

TABLE 3 : RANGE FINDING TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : WP2 uvrA

Treatment	Colony counts	Mean
Negative control	30	
CGA 62826 tech.:		
20.58 µg/plate	30	
61.73 µg/plate	26	
185.19 µg/plate	25	
555.56 µg/plate	27	
1666.67 µg/plate	30	
5000.00 µg/plate	28	

Treatment	Remarks	Factor
Negative control	-	1.00
CGA 62826 tech.:		
20.58 µg/plate	-	1.00
61.73 µg/plate	-	0.87
185.19 µg/plate	-	0.83
555.56 µg/plate	-	0.90
1666.67 µg/plate	-	1.00
5000.00 µg/plate	-	0.93

TABLE 4 : RANGE FINDING TEST
Experiment without metabolic activation

Test number	:	**963102**
Experiment	:	**Original**
Test substance	:	**CGA 62826 tech.**
Batch	:	**RV-1592/4**
Strain	:	**WP2 uvrA**

Treatment	Colony counts	Mean
Negative control	17	
CGA 62826 tech.:		
20.58 µg/plate	25	
61.73 µg/plate	16	
185.19 µg/plate	24	
555.56 µg/plate	15	
1666.67 µg/plate	24	
5000.00 µg/plate	27	

Treatment	Remarks	Factor
Negative control	-	1.00
CGA 62826 tech.:		
20.58 µg/plate	-	1.47
61.73 µg/plate	-	0.94
185.19 µg/plate	-	1.41
555.56 µg/plate	-	0.88
1666.67 µg/plate	-	1.41
5000.00 µg/plate	-	1.59

TABLE 5 : SUMMARY OF THE MUTAGENICITY EXPERIMENTS
Experiments with metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	128.33	TA 1535	Negative control	11.33
	312.50 µg/plate	125.67		312.50 µg/plate	15.33
	625.00 µg/plate	138.33		625.00 µg/plate	12.00
	1250.00 µg/plate	151.67		1250.00 µg/plate	9.00
	2500.00 µg/plate	143.67		2500.00 µg/plate	14.00
	5000.00 µg/plate	144.00		5000.00 µg/plate	13.67
	Positive control	2343.67		Positive control	247.33
WP2 uvrA	Negative control	20.67	TA 98	Negative control	21.33
	312.50 µg/plate	20.67		312.50 µg/plate	24.33
	625.00 µg/plate	20.33		625.00 µg/plate	22.00
	1250.00 µg/plate	18.00		1250.00 µg/plate	23.33
	2500.00 µg/plate	22.33		2500.00 µg/plate	24.33
	5000.00 µg/plate	15.67		5000.00 µg/plate	24.67
	Positive control	1067.00		Positive control	1202.67
TA 1537	Negative control	10.00	TA 102	Negative control	295.67
	312.50 µg/plate	8.67		312.50 µg/plate	319.67
	625.00 µg/plate	10.33		625.00 µg/plate	288.67
	1250.00 µg/plate	8.00		1250.00 µg/plate	309.00
	2500.00 µg/plate	7.67		2500.00 µg/plate	277.00
	5000.00 µg/plate	7.67		5000.00 µg/plate	262.67
	Positive control	197.33		Positive control	2180.00

TABLE 6 : SUMMARY OF THE MUTAGENICITY EXPERIMENTS
Experiments without metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	139.00	TA 1535	Negative control	11.33
	312.50 µg/plate	135.00		312.50 µg/plate	13.33
	625.00 µg/plate	133.00		625.00 µg/plate	11.33
	1250.00 µg/plate	106.67		1250.00 µg/plate	17.67
	2500.00 µg/plate	132.67		2500.00 µg/plate	17.67
	5000.00 µg/plate	125.33		5000.00 µg/plate	11.00
	Positive control	1168.00		Positive control	861.67
WP2 uvrA	Negative control	20.00	TA 98	Negative control	16.33
	312.50 µg/plate	18.33		312.50 µg/plate	20.00
	625.00 µg/plate	18.00		625.00 µg/plate	15.33
	1250.00 µg/plate	18.00		1250.00 µg/plate	17.33
	2500.00 µg/plate	18.67		2500.00 µg/plate	15.67
	5000.00 µg/plate	14.00		5000.00 µg/plate	21.00
	Positive control	488.67		Positive control	522.00
TA 1537	Negative control	8.33	TA 102	Negative control	274.67
	312.50 µg/plate	7.33		312.50 µg/plate	291.67
	625.00 µg/plate	9.67		625.00 µg/plate	276.33
	1250.00 µg/plate	8.00		1250.00 µg/plate	274.67
	2500.00 µg/plate	10.00		2500.00 µg/plate	235.33
	5000.00 µg/plate	4.00		5000.00 µg/plate	238.00
	Positive control	1114.33		Positive control	1245.67

TABLE 7 : SUMMARY OF THE MUTAGENICITY EXPERIMENTS
Experiments with metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	137.00	TA 1535	Negative control	11.67
	312.50 µg/plate	120.33		312.50 µg/plate	16.33
	625.00 µg/plate	124.67		625.00 µg/plate	17.67
	1250.00 µg/plate	131.00		1250.00 µg/plate	15.67
	2500.00 µg/plate	129.67		2500.00 µg/plate	14.33
	5000.00 µg/plate	122.00		5000.00 µg/plate	14.67
	Positive control	660.33		Positive control	353.67
WP2 uvrA	Negative control	26.67	TA 98	Negative control	43.67
	312.50 µg/plate	26.33		312.50 µg/plate	42.00
	625.00 µg/plate	30.00		625.00 µg/plate	44.33
	1250.00 µg/plate	25.00		1250.00 µg/plate	42.67
	2500.00 µg/plate	25.67		2500.00 µg/plate	41.33
	5000.00 µg/plate	19.33		5000.00 µg/plate	41.67
	Positive control	258.33		Positive control	625.33
TA 1537	Negative control	12.67	TA 102	Negative control	284.67
	312.50 µg/plate	11.67		312.50 µg/plate	272.67
	625.00 µg/plate	12.00		625.00 µg/plate	257.67
	1250.00 µg/plate	13.33		1250.00 µg/plate	249.67
	2500.00 µg/plate	13.67		2500.00 µg/plate	244.00
	5000.00 µg/plate	14.33		5000.00 µg/plate	195.67
	Positive control	122.33		Positive control	1107.00

TABLE 8 : SUMMARY OF THE MUTAGENICITY EXPERIMENTS
Experiments without metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	134.00	TA 1535	Negative control	19.33
	312.50 µg/plate	119.67		312.50 µg/plate	19.33
	625.00 µg/plate	127.67		625.00 µg/plate	15.00
	1250.00 µg/plate	121.67		1250.00 µg/plate	14.33
	2500.00 µg/plate	115.33		2500.00 µg/plate	15.33
	5000.00 µg/plate	102.33		5000.00 µg/plate	13.00
	Positive control	1180.33		Positive control	771.33
WP2 uvrA	Negative control	25.33	TA 98	Negative control	26.67
	312.50 µg/plate	24.67		312.50 µg/plate	27.33
	625.00 µg/plate	21.67		625.00 µg/plate	24.00
	1250.00 µg/plate	19.67		1250.00 µg/plate	26.00
	2500.00 µg/plate	22.67		2500.00 µg/plate	28.00
	5000.00 µg/plate	22.33		5000.00 µg/plate	30.33
	Positive control	482.00		Positive control	413.67
TA 1537	Negative control	9.33	TA 102	Negative control	258.00
	312.50 µg/plate	10.33		312.50 µg/plate	231.33
	625.00 µg/plate	8.33		625.00 µg/plate	235.00
	1250.00 µg/plate	8.00		1250.00 µg/plate	173.00
	2500.00 µg/plate	8.33		2500.00 µg/plate	135.33
	5000.00 µg/plate	6.67		5000.00 µg/plate	22.33
	Positive control	924.33		Positive control	1234.67

TABLE 9 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 100

Treatment	Colony counts			Mean
Negative control	115	137	133	128.33
CGA 62826 tech.:				
312.50 µg/plate	105	128	144	125.67
625.00 µg/plate	125	151	139	138.33
1250.00 µg/plate	136	150	169	151.67
2500.00 µg/plate	125	146	160	143.67
5000.00 µg/plate	124	148	160	144.00
2-Aminoanthracene 1.50 µg/plate	2372	2569	2090	2343.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.98
625.00 µg/plate	-	-	-	1.08
1250.00 µg/plate	-	-	-	1.18
2500.00 µg/plate	-	-	-	1.12
5000.00 µg/plate	-	-	-	1.12
2-Aminoanthracene	-	-	-	18.26

TABLE 10 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 1535

Treatment	Colony counts			Mean
Negative control	14	12	8	11.33
CGA 62826 tech.:				
312.50 µg/plate	12	15	19	15.33
625.00 µg/plate	16	10	10	12.00
1250.00 µg/plate	9	8	10	9.00
2500.00 µg/plate	13	16	13	14.00
5000.00 µg/plate	14	14	13	13.67
Cyclophosphamide 200.00 µg/plate	254	265	223	247.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	1.35
625.00 µg/plate	-	-	-	1.06
1250.00 µg/plate	-	-	-	0.79
2500.00 µg/plate	-	-	-	1.24
5000.00 µg/plate	-	-	-	1.21
Cyclophosphamide	-	-	-	21.82

TABLE 11 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : WP2 uvrA

Treatment	Colony counts			Mean
Negative control	21	23	18	20.67
CGA 62826 tech.:				
312.50 µg/plate	18	24	20	20.67
625.00 µg/plate	25	19	17	20.33
1250.00 µg/plate	15	13	26	18.00
2500.00 µg/plate	29	22	16	22.33
5000.00 µg/plate	15	16	16	15.67
2-Aminoanthracene 20.00 µg/plate	1129	989	1083	1067.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	1.00
625.00 µg/plate	-	-	-	0.98
1250.00 µg/plate	-	-	-	0.87
2500.00 µg/plate	-	-	-	1.08
5000.00 µg/plate	-	-	-	0.76
2-Aminoanthracene	-	-	-	51.63

TABLE 12 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 98

Treatment	Colony counts			Mean
Negative control	23	24	17	21.33
CGA 62826 tech.:				
312.50 µg/plate	22	24	27	24.33
625.00 µg/plate	24	22	20	22.00
1250.00 µg/plate	19	23	28	23.33
2500.00 µg/plate	26	21	26	24.33
5000.00 µg/plate	24	28	22	24.67
2-Aminoanthracene 1.50 µg/plate	1335	1304	969	1202.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	1.14
625.00 µg/plate	-	-	-	1.03
1250.00 µg/plate	-	-	-	1.09
2500.00 µg/plate	-	-	-	1.14
5000.00 µg/plate	-	-	-	1.16
2-Aminoanthracene	-	-	-	56.37

TABLE 13 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 1537

Treatment	Colony counts			Mean
Negative control	11	7	12	10.00
CGA 62826 tech.:				
312.50 µg/plate	10	10	6	8.67
625.00 µg/plate	5	14	12	10.33
1250.00 µg/plate	5	12	7	8.00
2500.00 µg/plate	8	6	9	7.67
5000.00 µg/plate	9	4	10	7.67
2-Aminoanthracene 1.50 µg/plate	201	192	199	197.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.87
625.00 µg/plate	-	-	-	1.03
1250.00 µg/plate	-	-	-	0.80
2500.00 µg/plate	-	-	-	0.77
5000.00 µg/plate	-	-	-	0.77
2-Aminoanthracene	-	-	-	19.73

TABLE 14 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 102

Treatment	Colony counts			Mean
Negative control	294	290	303	295.67
CGA 62826 tech.:				
312.50 µg/plate	341	316	302	319.67
625.00 µg/plate	266	284	316	288.67
1250.00 µg/plate	307	318	302	309.00
2500.00 µg/plate	236	295	300	277.00
5000.00 µg/plate	271	261	256	262.67
2-Aminoanthracene 5.00 µg/plate	2241	2264	2035	2180.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	1.08
625.00 µg/plate	-	-	-	0.98
1250.00 µg/plate	-	-	-	1.05
2500.00 µg/plate	-	-	-	0.94
5000.00 µg/plate	-	-	-	0.89
2-Aminoanthracene	-	-	-	7.37

TABLE 15 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 100

Treatment	Colony counts			Mean
Negative control	136	125	156	139.00
CGA 62826 tech.:				
312.50 µg/plate	122	151	132	135.00
625.00 µg/plate	140	121	138	133.00
1250.00 µg/plate	105	98	117	106.67
2500.00 µg/plate	127	133	138	132.67
5000.00 µg/plate	141	115	120	125.33
Sodium azide 2.00 µg/plate	1148	1178	1178	1168.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.97
625.00 µg/plate	-	-	-	0.96
1250.00 µg/plate	-	-	-	0.77
2500.00 µg/plate	-	-	-	0.95
5000.00 µg/plate	-	-	-	0.90
Sodium azide	-	-	-	8.40

TABLE 16 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 1535

Treatment	Colony counts			Mean
Negative control	11	8	15	11.33
CGA 62826 tech.:				
312.50 µg/plate	17	12	11	13.33
625.00 µg/plate	9	13	12	11.33
1250.00 µg/plate	16	16	21	17.67
2500.00 µg/plate	19	17	17	17.67
5000.00 µg/plate	9	14	10	11.00
Sodium azide 2.00 µg/plate	835	895	855	861.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	1.18
625.00 µg/plate	-	-	-	1.00
1250.00 µg/plate	-	-	-	1.56
2500.00 µg/plate	-	-	-	1.56
5000.00 µg/plate	-	-	-	0.97
Sodium azide	-	-	-	76.03

TABLE 17 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : WP2 uvrA

Treatment	Colony counts			Mean
Negative control	24	16	20	20.00
CGA 62826 tech.:				
312.50 µg/plate	22	13	20	18.33
625.00 µg/plate	26	11	17	18.00
1250.00 µg/plate	21	16	17	18.00
2500.00 µg/plate	20	21	15	18.67
5000.00 µg/plate	13	11	18	14.00
4-NQO 2.00 µg/plate	510	497	459	488.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.92
625.00 µg/plate	-	-	-	0.90
1250.00 µg/plate	-	-	-	0.90
2500.00 µg/plate	-	-	-	0.93
5000.00 µg/plate	-	-	-	0.70
4-NQO	-	-	-	24.43

TABLE 18 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 98

Treatment	Colony counts			Mean
Negative control	18	16	15	16.33
CGA 62826 tech.:				
312.50 µg/plate	23	18	19	20.00
625.00 µg/plate	15	16	15	15.33
1250.00 µg/plate	20	15	17	17.33
2500.00 µg/plate	10	19	18	15.67
5000.00 µg/plate	20	17	26	21.00
2-Nitrofluorene 5.00 µg/plate	516	521	529	522.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	1.22
625.00 µg/plate	-	-	-	0.94
1250.00 µg/plate	-	-	-	1.06
2500.00 µg/plate	-	-	-	0.96
5000.00 µg/plate	-	-	-	1.29
2-Nitrofluorene	-	-	-	31.96

TABLE 19 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 1537

Treatment	Colony counts			Mean
Negative control	8	8	9	8.33
CGA 62826 tech.:				
312.50 µg/plate	7	5	10	7.33
625.00 µg/plate	13	12	4	9.67
1250.00 µg/plate	7	4	13	8.00
2500.00 µg/plate	8	10	12	10.00
5000.00 µg/plate	1	7	4	4.00
9-Aminoacridine 80.00 µg/plate	1200	1225	918	1114.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.88
625.00 µg/plate	-	-	-	1.16
1250.00 µg/plate	-	-	-	0.96
2500.00 µg/plate	-	-	-	1.20
5000.00 µg/plate	-	-	-	0.48
9-Aminoacridine	-	-	-	133.72

TABLE 20 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Original
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 102

Treatment	Colony counts			Mean
Negative control	244	288	292	274.67
CGA 62826 tech.:				
312.50 µg/plate	284	313	278	291.67
625.00 µg/plate	268	283	278	276.33
1250.00 µg/plate	261	269	294	274.67
2500.00 µg/plate	213	236	257	235.33
5000.00 µg/plate	234	264	216	238.00
Mitomycin-C 0.50 µg/plate	1239	1267	1231	1245.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	1.06
625.00 µg/plate	-	-	-	1.01
1250.00 µg/plate	-	-	-	1.00
2500.00 µg/plate	-	-	-	0.86
5000.00 µg/plate	-	-	-	0.87
Mitomycin-C	-	-	-	4.54

TABLE 21 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number	:	**963102**
Experiment	:	**Confirmatory**
Test substance	:	**CGA 62826 tech.**
Batch	:	**RV-1592/4**
Strain	:	**TA 100**

Treatment	**Colony counts**			**Mean**
Negative control	126	133	152	137.00
CGA 62826 tech.:				
312.50 µg/plate	124	102	135	120.33
625.00 µg/plate	140	109	125	124.67
1250.00 µg/plate	146	121	126	131.00
2500.00 µg/plate	123	122	144	129.67
5000.00 µg/plate	108	129	129	122.00
2-Aminoanthracene 1.50 µg/plate	768	785	428	660.33

Treatment	**Remarks**			**Factor**
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.88
625.00 µg/plate	-	-	-	0.91
1250.00 µg/plate	-	-	-	0.96
2500.00 µg/plate	-	-	-	0.95
5000.00 µg/plate	-	-	-	0.89
2-Aminoanthracene	-	-	-	4.82

TABLE 22 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 1535

Treatment	Colony counts			Mean
Negative control	14	13	8	11.67
CGA 62826 tech.:				
312.50 µg/plate	14	20	15	16.33
625.00 µg/plate	12	19	22	17.67
1250.00 µg/plate	10	14	23	15.67
2500.00 µg/plate	13	17	13	14.33
5000.00 µg/plate	12	11	21	14.67
Cyclophosphamide 200.00 µg/plate	378	291	392	353.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	1.40
625.00 µg/plate	-	-	-	1.51
1250.00 µg/plate	-	-	-	1.34
2500.00 µg/plate	-	-	-	1.23
5000.00 µg/plate	-	-	-	1.26
Cyclophosphamide	-	-	-	30.31

TABLE 23 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : WP2 uvrA

Treatment	Colony counts			Mean
Negative control	26	25	29	26.67
CGA 62826 tech.:				
312.50 µg/plate	25	26	28	26.33
625.00 µg/plate	27	26	37	30.00
1250.00 µg/plate	21	26	28	25.00
2500.00 µg/plate	23	21	33	25.67
5000.00 µg/plate	22	19	17	19.33
2-Aminoanthracene 20.00 µg/plate	337	256	182	258.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.99
625.00 µg/plate	-	-	-	1.13
1250.00 µg/plate	-	-	-	0.94
2500.00 µg/plate	-	-	-	0.96
5000.00 µg/plate	-	-	-	0.73
2-Aminoanthracene	-	-	-	9.69

TABLE 24 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 98

Treatment	Colony counts			Mean
Negative control	48	38	45	43.67
CGA 62826 tech.:				
312.50 µg/plate	31	52	43	42.00
625.00 µg/plate	47	49	37	44.33
1250.00 µg/plate	42	33	53	42.67
2500.00 µg/plate	45	38	41	41.33
5000.00 µg/plate	41	39	45	41.67
2-Aminoanthracene 1.50 µg/plate	651	627	598	625.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.96
625.00 µg/plate	-	-	-	1.02
1250.00 µg/plate	-	-	-	0.98
2500.00 µg/plate	-	-	-	0.95
5000.00 µg/plate	-	-	-	0.95
2-Aminoanthracene	-	-	-	14.32

TABLE 25 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 1537

Treatment	Colony counts			Mean
Negative control	13	11	14	12.67
CGA 62826 tech.:				
312.50 µg/plate	9	14	12	11.67
625.00 µg/plate	16	10	10	12.00
1250.00 µg/plate	13	17	10	13.33
2500.00 µg/plate	15	14	12	13.67
5000.00 µg/plate	14	11	18	14.33
2-Aminoanthracene 1.50 µg/plate	123	121	123	122.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.92
625.00 µg/plate	-	-	-	0.95
1250.00 µg/plate	-	-	-	1.05
2500.00 µg/plate	-	-	-	1.08
5000.00 µg/plate	-	-	-	1.13
2-Aminoanthracene	-	-	-	9.66

TABLE 26 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 102

Treatment	Colony counts			Mean
Negative control	303	260	291	284.67
CGA 62826 tech.:				
312.50 µg/plate	284	268	266	272.67
625.00 µg/plate	258	257	258	257.67
1250.00 µg/plate	244	270	235	249.67
2500.00 µg/plate	243	242	247	244.00
5000.00 µg/plate	222	180	185	195.67
2-Aminoanthracene 4.00 µg/plate	1406	1057	858	1107.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.96
625.00 µg/plate	-	-	-	0.91
1250.00 µg/plate	-	-	-	0.88
2500.00 µg/plate	-	-	-	0.86
5000.00 µg/plate	-	-	-	0.69
2-Aminoanthracene	-	-	-	3.89

TABLE 27 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 100

Treatment	Colony counts			Mean
Negative control	137	133	132	134.00
CGA 62826 tech.:				
312.50 µg/plate	104	132	123	119.67
625.00 µg/plate	136	126	121	127.67
1250.00 µg/plate	115	126	124	121.67
2500.00 µg/plate	110	121	115	115.33
5000.00 µg/plate	101	127	79	102.33
Sodium azide 2.00 µg/plate	1131	1205	1205	1180.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.89
625.00 µg/plate	-	-	-	0.95
1250.00 µg/plate	-	-	-	0.91
2500.00 µg/plate	-	-	-	0.86
5000.00 µg/plate	-	-	-	0.76
Sodium azide	-	-	-	8.81

TABLE 28 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 1535

Treatment	Colony counts			Mean
Negative control	19	22	17	19.33
CGA 62826 tech.:				
312.50 µg/plate	21	24	13	19.33
625.00 µg/plate	14	15	16	15.00
1250.00 µg/plate	17	18	8	14.33
2500.00 µg/plate	17	13	16	15.33
5000.00 µg/plate	12	13	14	13.00
Sodium azide 2.00 µg/plate	784	792	738	771.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	1.00
625.00 µg/plate	-	-	-	0.78
1250.00 µg/plate	-	-	-	0.74
2500.00 µg/plate	-	-	-	0.79
5000.00 µg/plate	-	-	-	0.67
Sodium azide	-	-	-	39.90

TABLE 29 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : WP2 uvrA

Treatment	Colony counts			Mean
Negative control	26	28	22	25.33
CGA 62826 tech.:				
312.50 µg/plate	32	19	23	24.67
625.00 µg/plate	26	17	22	21.67
1250.00 µg/plate	22	16	21	19.67
2500.00 µg/plate	23	28	17	22.67
5000.00 µg/plate	23	23	21	22.33
4-NQO 2.00 µg/plate	417	500	529	482.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.97
625.00 µg/plate	-	-	-	0.86
1250.00 µg/plate	-	-	-	0.78
2500.00 µg/plate	-	-	-	0.89
5000.00 µg/plate	-	-	-	0.88
4-NQO	-	-	-	19.03

TABLE 30 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 98

Treatment	Colony counts			Mean
Negative control	29	20	31	26.67
CGA 62826 tech.:				
312.50 µg/plate	32	25	25	27.33
625.00 µg/plate	28	15	29	24.00
1250.00 µg/plate	24	27	27	26.00
2500.00 µg/plate	36	27	21	28.00
5000.00 µg/plate	31	29	31	30.33
2-Nitrofluorene 5.00 µg/plate	439	378	424	413.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	1.03
625.00 µg/plate	-	-	-	0.90
1250.00 µg/plate	-	-	-	0.98
2500.00 µg/plate	-	-	-	1.05
5000.00 µg/plate	-	-	-	1.14
2-Nitrofluorene	-	-	-	15.51

TABLE 31 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 1537

Treatment	Colony counts			Mean
Negative control	12	7	9	9.33
CGA 62826 tech.:				
312.50 µg/plate	10	9	12	10.33
625.00 µg/plate	8	9	8	8.33
1250.00 µg/plate	11	6	7	8.00
2500.00 µg/plate	7	6	12	8.33
5000.00 µg/plate	6	11	3	6.67
9-Aminoacridine 80.00 µg/plate	901	908	964	924.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	1.11
625.00 µg/plate	-	-	-	0.89
1250.00 µg/plate	-	-	-	0.86
2500.00 µg/plate	-	-	-	0.89
5000.00 µg/plate	-	-	-	0.71
9-Aminoacridine	-	-	-	99.04

TABLE 32 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963102
Experiment : Confirmatory
Test substance : CGA 62826 tech.
Batch : RV-1592/4
Strain : TA 102

Treatment	Colony counts			Mean
Negative control	260	254	260	258.00
CGA 62826 tech.:				
312.50 µg/plate	268	255	171	231.33
625.00 µg/plate	261	232	212	235.00
1250.00 µg/plate	221	160	138	173.00
2500.00 µg/plate	138	134	134	135.33
5000.00 µg/plate	44	20	3	22.33
Mitomycin-C 0.50 µg/plate	1220	1220	1264	1234.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 62826 tech.:				
312.50 µg/plate	-	-	-	0.90
625.00 µg/plate	-	-	-	0.91
1250.00 µg/plate	-	-	-	0.67
2500.00 µg/plate	-	-	-	0.52
5000.00 µg/plate	-	-	-	0.09
Mitomycin-C	-	-	-	4.79

SALMONELLA AND ESCHERICHIA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

SUMMARIZED REPORT TO 963102

CGA 62826 tech. (metabolite of CGA 48988)

Report of Result of Mutagenicity Test using Microorganisms

1. General Item

Name of the new chemical substance (IUPAC nomenclature)			
Other name	CGA 62826 tech. (metabolite of CGA 48988)		
Structural formula or rational formula (or outline of manu-facturing method, in case both are unknown)			
Purity of the new chemical substance tested	100%	Lot of the chemical substance tested	RV-1592/4
Name and concentration of impurities			
CAS number		Vapor pressure	
Molecular weight		Partition coefficient	
Melting point		Appearance at ordinary temperature	Solid
Boiling point			
Stability			

	Solvent	Solubility	Solvent	Solubility
Solubility in solvent	Water		DMSO	50 mg/ml
	Acetone			

2. Tester Strains

(1) Procurement

Strains	Obtained from	Date obtained	Date of confirming genotypes of the stored lot used in the experiment
TA 98	Prof. [redacted]	January 1983	August 07, 1996
TA 100	Dr. [redacted] (Hoffmann-La Roche)	June 1986	August 07, 1996
TA 102	Prof. [redacted]	January 1983	August 07, 1996
TA 1535	Prof. [redacted]	January 1983	August 07, 1996
TA 1537	Dr. [redacted] (Hoffmann-La Roche)	July 1994	August 07, 1996
E. coli WP2 uvrA	National Collection of Industrial Bacteria, Aberdeen, Scotland	April 1977	August 07, 1996

(2) Storage (The applicable number is darkly marked)

Method of storage	1. Frozen in small volume	2. Frozen in large volume	3. Others
Storage temperature	-80°C		
Composition	Bacterial suspension: 9.25 ml DMSO: 0.75 ml		

3. S9 Mix

(1) Source of S9 (The applicable number is darkly marked)

Made in-house or purchased	1. Made in-house	2. Purchased
Prepared on	December 14, 1995	
Lot No. (if purchased)		
Storage temperature	-80°C	

(2) Preparation of S9

Animal used		Inducing substance	
Species, Strain	Rat, Tif:RAIF [SPF]	Name	Aroclor 1254
Sex	Male	Administration method	i.p.
Age (in weeks)	Approx. 7 weeks	Administration period and amount	Single application 5 days prior sacrifice
Weight	169 to 186 g	(g/kg body weight)	0.5 g/kg in sesame oil

(3) Composition of S9 Mix

Constituents	Amount in 1 ml S9 mix	Constituents	Amount in 1 ml S9 mix
S9	0.1 ml		
$MgCl_2$	8.0 µmol	NADP	4.0 µmol
KCl	33.0 µmol	Na-phosphate buffer (pH 7.4)	100.0 µmol
Glucose-6-phosphate	5.0 µmol		
Glucose-6-phosphate dehydrogenase			

4. Positive Control and its Solvent (The applicable number is darkly marked)

Substance		Supplier	Lot No.	Grade	Purity %	Solvent
Positive control	9-Aminoacridine	Fluka Switzerland	266691 688	purum	98.5	DMSO
	2-Aminoanthracene	Sigma USA	35H2507	practicum	*	DMSO
	Cyclophosphamide	NBS Biologic., GB	930K/60949/1	*	97	Bidistilled water
	Sodium azide	Fluka Switzerland	347911/1	purum	>99.0	Bidistilled water
	4-Nitroquinoline (4-NQO)	Fluka Switzerland	219741/1 1192	purum	>97.0	DMSO
	2-Nitrofluorene	Merck Germany	1296187	p.a.	>98.0	DMSO
	Mitomycin-C	Syntex Pharm Switzerland	942191	*	*	Bidistilled water
Preparation and storage etc. of the solution of the positive control		1. Prepared in use				
		2. Distributed and stored (storage temperature -20°C)				
		3. Numbers 1&2 §				

* No data available
§ Cyclophosphamide prepared in use. From all other controls stored samples were used

5. Preparation of the Solution of the Test Substance (The applicable number is darkly marked)

Solvent used	Name	Supplier	Lot No.	Grade	Purity %
	DMSO	Merck, Germany	K222948315 43/K2260543 1614	puriss p.a.	99.5%
Stability of the test substance in the solvent					
Rationale for selection of the solvent	Solubility				
Method of suspension etc. when test substance is difficult to dissolve					
Storage period and temperature of the solution from preparation until use	About half an hour In case aqueous solutions are used they are stored on ice at 4°C All other solutions are stored at room temperature in the dark				
Correction of purity	Yes				
	No				

6. Conditions of Pre-culture, etc.

(1) Condition

Nutrient broth	Name	Manufacture	Lot No.
	Nutrient broth No. 2	Oxoid Ltd., Basingstoke, England	07455907
Period of pre-culture	8 hours		
Storage period/temperature from inoculation of the tester strains until initiation of incubation with shaking	About 8 hours at 12°C		
Storage period/temperature from completion of incubation until use	1 hour at room temperature		
Model and manufacturer of shaking incubator	Orbital shaker IKA, Janke & Kunkel, Germany		
Method of shaking (Procedure, times of shaking, etc.)	Orbital shaking at 37°C, 130-140 rounds per minute		
Container for incubation (shape, volume)	300 ml erlenmeyer flask		
Volume of medium	60 ml	Volume of the tester strain inoculated	60 µl from a frozen stock

(2) Density of Tester Strain Cultures at the Termination of Pre-culture (The applicable number is darkly marked)

		Base-pair substitution type				Frameshift type	
		TA 100	TA 1535	WP2 uvrA	TA 102	TA 98	TA 1537
Density $x10^9$/ml	Range finding study	1.61		4.05			
	Main study Orig./Conf.	1.70/1.56	1.34/2.03	3.08/3.55	1.81/2.30	1.55/2.00	1.29/1.30
Method of determination	1. Conversion from O.D. value						
	2. Dilution method						
	3. Others						

7. Agar Medium, etc.

(1) Top Agar

Agar	Name	Agar, bacteriological grade
	Manufacturer	GIBCO Ltd., Paisley, Scotland
	Lot No.	20E9061B

(2) Minimum Glucose Agar Plate Medium (The applicable number is darkly marked)

Made in-house or purchased	1. Made in-house
	2. Purchased
Prepared on	2-10 days before start of the experiment
Lot No. (if purchased)	
Name/Manufacturer/Lot No. of agar used	GIBCO Ltd., Paisley, Scotland 20E9061B

8. Sterility Test

	Bacterial growth other than those used for test	
Test substance solution	Yes	No
S9 Mix	Yes	No

9. Test Method

		Plate method	Pre-incubation method
Composition	Bacterial suspension	0.1 ml	0.1 ml
	Test substance solution	0.1 ml	0.1 ml
	Na-phosphate buffer	0.5 ml	
	S9 Mix (in case of metabolic activation method)	0.5 ml	0.5 ml
	Top agar solution	2.0 ml	2.0 ml
Pre-incubation	Temperature		about 37°C
	Time		about 30 minutes
Incubation	Temperature	about 37°C	about 37°C
	Time	about 48 hours	about 48 hours

10. Method of Counting the Number of Colonies (The applicable number is darkly marked)

Method of counting	1. Manual
	2. With colony counter
Rationale for using the methods of nos. 1 and 2	Method is exact, efficient and economic
Name, model and manufacturer of colony counter	Artek Colony Counter, Model 880 Artek Systems Corporation, USA
Method for correction	1. Not corrected
	2. Correction with area
	3. Correction for numbers counted out
	4. Nos. 2 & 3

11. Test result

(1) Test results should be reported on the attached form
(2) Judgement of the result

Judgement	Positive
	Negative

Reason for judgement and referential matters:
There was no increase in the number of back-mutant colonies in comparison with the negative control at any tested concentration of CGA 62826 tech.

12. Others

Testing Institution	**Name**	Novartis Crop Protection Inc., Genetic Toxicology		
	Address	CH-4002 Basle, Switzerland, Tel.: 41 61/697 11 11		
Administrator	**Name**	Dr. 5.1.2.e Woo	**Signature**	5.1.2.e Woo
			Final education career and specialized field	Dr. phil. II, Zoology University of Basle
Individual Responsible for Archive Storage	**Name**	Mrs. 5.1.2.e Woo	**Signature**	5.1.2.e Woo
Individual Responsible for Quality Assurance	**Name**	Mr. 5.1.2.e Woo 5.1.2.e Woo	**Signature**	
Study Director	**Name**	Mr. 5.1.2.e Woo	**Signature**	
	Years of experience	26 years	**Final education career and specialized field**	See Appendix
Personnel engaged in study	**Name**	Mrs. 5.1.2.e Woo	**Signature**	5.1.2.e Woo
	Years of experience	6 years	**Final education career and specialized field**	See Appendix
Personnel engaged in study	**Name**	Mrs. 5.1.2.e Woo	**Signature**	5.1.2.e Woo
	Years of experience	19 years	**Final education career and specialized field**	See Appendix
Test dates	Study initiation date:	September 04, 1996		
	Experimental start date:	September 18, 1996		
	Experimental termination date:	October 17, 1996		
	Study termination date:	January 10, 1997		
Test number	963102			

Remarks

1. "Stability". Fill in the stability for water, other solvents, heat, light, etc.
2. "Vapor pressure". Fill in the vapor pressure of the test substance at 25°C.
3. "Solubility". Fill in such information as water-soluble, soluble in oil.
4. "Degree of solubility". Fill in the solubility at 25°C for each solvent.
5. "Partition coefficient": Fill in the value at 37°C and the name of the solvent used for the measurement.
6. "Years of experience". Fill in the years of experience performing mutagenicity test.
7. "Reason for judgement and referential matters". Fill in the opinion of the Study Director of the test results.
8. "Administrator", "Study Director" and "Personnel engaged in a study" are defined in paragraph 2 of Annex 3, "Standards to be observed, Mutagenicity Testing Institutions".

Table of Test Results

(Original experiment)

Name of Test Substance: CGA 62826 tech.

With(+) or without(-) S9 Mix	Test substance concentration (µg/plate)	Number of revertants (number of colonies/plate)					
		Base-pair substitution type				Frameshift type	
		TA 100	TA 1535	WP2 uvrA	TA 102	TA 98	TA 1537
S9 Mix (+)	Solvent control	115 137 133 (128)	14 12 8 (11)	21 23 18 (21)	294 290 303 (296)	23 24 17 (21)	11 7 12 (10)
	312.50	105 128 144 (126)	12 15 19 (15)	18 24 20 (21)	341 316 302 (320)	22 24 27 (24)	10 10 6 (9)
	625.00	125 151 139 (138)	16 10 10 (12)	25 19 17 (20)	266 284 316 (289)	24 22 20 (22)	5 14 12 (10)
	1250.00	136 150 169 (152)	9 8 10 (9)	15 13 26 (18)	307 318 302 (309)	19 23 28 (23)	5 12 7 (8)
	2500.00	125 146 160 (144)	13 16 13 (14)	29 22 16 (22)	236 295 300 (277)	26 21 26 (24)	8 6 9 (8)
	5000.00	124 148 160 (144)	14 14 13 (14)	15 16 16 (16)	271 261 256 (263)	24 28 22 (25)	9 4 10 (8)
S9 Mix (-)	Solvent control	136 125 156 (139)	11 8 15 (11)	24 16 20 (20)	244 288 292 (275)	18 16 15 (16)	8 8 9 (8)
	312.50	122 151 132 (135)	17 12 11 (13)	22 13 20 (18)	284 313 278 (292)	23 18 19 (20)	7 5 10 (7)
	625.00	140 121 138 (133)	9 13 12 (11)	26 11 17 (18)	268 283 278 (276)	15 16 15 (15)	13 12 4 (10)
	1250.00	105 98 117 (107)	16 16 21 (18)	21 16 17 (18)	261 269 294 (275)	20 15 17 (17)	7 4 13 (8)
	2500.00	127 133 138 (133)	19 17 17 (18)	20 21 15 (19)	213 236 257 (235)	10 19 18 (16)	8 10 12 (10)
	5000.00	141 115 120 (125)	9 14 10 (11)	13 11 18 (14)	234 264 216 (238)	20 17 26 (21)	1 7 4 (4)*
Positive control requiring S9 Mix	Name	2-Amino-anthracene	Cyclo-phosphamide	2-Amino-anthracene	2-Amino-anthracene	2-Amino-anthracene	2-Amino-anthracene
	Concentration (ug/plate)	1.50	200.00	20.00	5.00	1.50	1.50
	Number of colonies/plate	2372 2569 2090 (2344)	254 265 223 (247)	1129 989 1083 (1067)	2241 2264 2035 (2180)	1335 1304 969 (1203)	201 192 199 (197)
Positive control not requiring S9 Mix	Name	Sodium azide	Sodium azide	4-NQO	Mitomycin-C	2-Nitro-fluorene	9-Amino-acridine
	Concentration (ug/plate)	2.00	2.00	2.00	0.50	5.00	80.00
	Number of colonies/plate	1148 1178 1178 (1168)	835 895 855 (862)	510 497 459 (489)	1239 1267 1231 (1246)	516 521 529 (522)	1200 1225 918 (1114)

Notes:
1. When inhibition is found against growth of the bacteria, mark the applicable value with an asterix
2. Fill the average number of colonies in each concentration in the ( )
3. "Number of revertants"-Fill in the observed value and average value in order beginning with low concentration of the test substance

Table of Test Results

(Confirmatory experiment)

Name of Test Substance: CGA 62826 tech.

With(+) or without(-) S9 Mix	Test substance concentration (µg/plate)	Number of revertants (number of colonies/plate)					
		Base-pair substitution type				Frameshift type	
		TA 100	TA 1535	WP2 uvrA	TA 102	TA 98	TA 1537
S9 Mix (+)	Solvent control	126 133 152 (137)	14 13 8 (12)	26 25 29 (27)	303 260 291 (285)	48 38 45 (44)	13 11 14 (13)
	312.50	124 102 135 (120)	14 20 15 (16)	25 26 28 (26)	284 268 266 (273)	31 52 43 (42)	9 14 12 (12)
	625.00	140 109 125 (125)	12 19 22 (18)	27 26 37 (30)	258 257 258 (258)	47 49 37 (44)	16 10 10 (12)
	1250.00	146 121 126 (131)	10 14 23 (16)	21 26 28 (25)	244 270 235 (250)	42 33 53 (43)	13 17 10 (13)
	2500.00	123 122 144 (130)	13 17 13 (14)	23 21 33 (26)	243 242 247 (244)	45 38 41 (41)	15 14 12 (14)
	5000.00	108 129 129 (122)	12 11 21 (15)	22 19 17 (19)	222 180 185 (196)	41 39 45 (42)	14 11 18 (14)
S9 Mix (-)	Solvent control	137 133 132 (134)	19 22 17 (19)	26 28 22 (25)	260 254 260 (258)	29 20 31 (27)	12 7 9 (9)
	312.50	104 132 123 (120)	21 24 13 (19)	32 19 23 (25)	268 255 171 (231)	32 25 25 (27)	10 9 12 (10)
	625.00	136 126 121 (128)	14 15 16 (15)	26 17 22 (22)	261 232 212 (235)	28 15 29 (24)	8 9 8 (8)
	1250.00	115 126 124 (122)	17 18 8 (14)	22 16 21 (20)	221 160 138 (173)	24 27 27 (26)	11 6 7 (8)
	2500.00	110 121 115 (115)	17 13 16 (15)	23 28 17 (23)	138 134 134 (135)	36 27 21 (28)	7 6 12 (8)
	5000.00	101 127 79 (102)	12 13 14 (13)	23 23 21 (22)	44 20 3 (22)*	31 29 31 (30)	6 11 3 (7)
Positive control requiring S9 Mix	Name	2-Amino-anthracene	Cyclo-phosphamide	2-Amino-anthracene	2-Amino-anthracene	2-Amino-anthracene	2-Amino-anthracene
	Concentration (ug/plate)	1.50	200.00	20.00	4.00	1.50	1.50
	Number of colonies/plate	768 785 428 (660)	378 291 392 (354)	337 256 182 (258)	1406 1057 858 (1107)	651 627 598 (625)	123 121 123 (122)
Positive control not requiring S9 Mix	Name	Sodium azide	Sodium azide	4-NQO	Mitomycin-C	2-Nitro-fluorene	9-Amino-acridine
	Concentration (ug/plate)	2.00	2.00	2.00	0.50	5.00	80.00
	Number of colonies/plate	1131 1205 1205 (1180)	784 792 738 (771)	417 500 529 (482)	1220 1220 1264 (1235)	439 378 424 (414)	901 908 964 (924)

Notes:
1. When inhibition is found against growth of the bacteria, mark the applicable value with an asterix
2. Fill the average number of colonies in each concentration in the (   )
3. "Number of revertants"-Fill in the observed value and average value in order beginning with low concentration of the test substance

SALMONELLA AND ESCHERICHIA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

CGA 62826 tech. (metabolite of CGA 48988)

APPENDIX 1 TO THE REPORT 963102

PERSONAL RECORDS OF STAFF

(5 pages)

Appendix to the summarized report

Appendix to the summarized report

Appendix to the summarized report

Appendix to the summarized report

SALMONELLA AND ESCHERICHIA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

CGA 62826 tech. (metabolite of CGA 48988)

APPENDIX 2 TO THE REPORT 963102

REPORT OF ANALYTICAL DETERMINATIONS

(6 pages)

CIBA-GEIGY Limited

ANALYSIS DATA

Test No. : 963102
Repetition : original

QA requested : X yes no

Test System : **SALMONELLA AND ESCHERICHIA/LIVER-MICROSOME TEST**

Segment : X only one , first , last
Treatment : X only one , first , last

Test Substance : **CGA 62826 tech.**
Batch No. : **RV-1592/4**
Vehicle : **DMSO**
Date of Preparation : **October 03, 1996**

Purity % : **100 %**
Storage conditions : **- 20°C**

Performed by (Name):

5.1.2.e Woo

(Visa)

Study Director:

Mr. 5.1.2.e Woo, R-1058.3.42, Tel. 75257

10/14/96
(Date)

5.1.2.e Woo
(Signature)

Delivered to Toxicology/Cell Biology:

Date: 10/14/96 Name: 5.1.2.e Woo Signature: 5.1.2.e Woo

	Data from Genetic Toxicology		Data from Toxicology/Cell Biology	
	Samples Nominal values (µg/ml)	Vol. (ml)	Determined values (µg/ml)	% of Nominal values
	3125.0	5.0	3044 / 3060	97.4 / 97.9

Analysis method: A960038

This study was performed in compliance with Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986.

Principal Investigator: 5.1.2.e Woo
Zellbiologie PP 2.523
R - 1058.2.48 5.1.2.e Woo

Oct. 17, 1996
(Date)

5.1.2.e Woo
(Signature)

Approved by:

R - 1058.2.52 Zellbiologie PP 2.523

Oct 17, 1996
(Date)

5.1.2.e Woo
(Signature)

[Raw data will be stored in the archives of Toxicology/Cell Biology, CIBA-GEIGY Limited, Basle, Switzerland]

CIBA-GEIGY Limited

ANALYSIS DATA

Test No. : 963102
Repetition : confirmatory
QA requested : X yes no

Test System : SALMONELLA AND ESCHERICHIA/LIVER-MICROSOME TEST

Segment : X only one , first , last
Treatment : X only one , first , last

Test Substance : CGA 62826 tech.
Batch No. : RV-1592/4
Vehicle : DMSO
Purity % : 100 %
Storage conditions : - 20°C
Date of Preparation : October 09, 1996

Performed by (Name):
5.1.2.e Woo

5.1.2.e Woo
(Visa)

Study Director:
Mr. 5.1.2.e Woo, R-1058.3.42, Tel. 5.1.2.e Woo 7

10/14/96 5.1.2.e Woo
(Date) (Signature)

Delivered to Toxicology/Cell Biology:

Date: 10/14/96 Name: 5.1.2.e Woo Signature: 5.1.2.e Woo

	Data from Genetic Toxicology		Data from Toxicology/Cell Biology	
	Samples Nominal values (µg/ml)	Vol. (ml)	Determined values (µg/ml)	% of Nominal values
	3125.0	5.0	2908/2910	93.1/93.1

Analysis method: A960038

This study was performed in compliance with Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986.

Principal Investigator: 5.1.2.e Woo
Zellbiologie PP 2.523
R - 1058.2.48 © 7 21 02

Oct. 17, 1996 5.1.2.e Woo
(Date) (Signature)

Approved by: 5.1.2.e Woo
Zellbiologie PP 2.523
R - 1058.2.52 © 7 61 93

Oct 17, 1996 5.1.2.e Woo
(Date) (Signature)

[Raw data will be stored in the archives of Toxicology/Cell Biology, CIBA-GEIGY Limited, Basle, Switzerland]

Determination of CGA 62826 tech. in DMSO

Objective

Determination of the content of CGA 62826 tech. in DMSO by HPLC.

Method Evaluation:	Substance:	CGA 62826 tech.
	Batch:	RV-1592/4
	Matrix:	DMSO

Test Facility

CIBA-GEIGY Ltd., Toxicology/Cell Biology, CH-4002 Basel.

Principal Investigator:
5.1.2.e Woo Date/Signature: *October 15, 1996* 5.1.2.e Woo

Approved:
5.1.2.e Woo Signature: 5.1.2.e Woo

Abstract:

The sample is diluted according to SOP 2.7.4.58 and analysed by HPLC (Nucleosil C18, 5 µm, 125x4.6 mm; acetonitrile/0.1% aqueous phosphoric acid) with UV detection (212 nm).

Chemicals

Milipore SQS water (HPLC quality)
Acetonitrile (HPLC quality)
Dimethylsulfoxide (puriss, p.a.)
Phosphoric acid (suprapur)

Test Samples

After intensive shaking an aliquot of the samples to be analysed is diluted with acetonitrile to a final concentration among 1-100 µg/ml.

Reference Samples

Two stock solutions of about 20 mg CGA 62826 tech. dissolved in 25 ml DMSO are prepared. These stock solutions are diluted with acetonitrile to yield final concentrations in the range of the test samples (R1, R2).

HPLC Conditions

Pump:	SP8800 / SP P4000	
Detector:	Spectra Focus / Spectra 100, wavelenth: 212 nm	
Integrator:	SP4290 / PE Nelson Turbochrom	
Column:	Nucleosil C18, 5 µm, 125x4.6 mm	
Guard column:	Nucleosil C18, 5 µm, 20x4.6 mm	
Mobile phase:	A:	Acetonitril
	B:	0.1% aqueous phosphoric acid
Gradient:	0 - 5 min	30 - 70% A
Temperature:	Ambient	
Flow rate:	1.5 ml/min	
Injection volume:	20 µl	
Retention time:	approx. 3.8 min	
Analysis time:	approx. 5.0 min	

Injection Sequence

R1,R2,S...S,R1,R2

R1,R2:	Reference samples R1, R2
S:	Test samples

Calculation

The concentration of CGA 62826 tech. the test sample is calculated from the peak area in comparison with the mean peak area obtained with the reference solutions according to the following equation:

$$C_S = \frac{A_S \times C_R}{A_R} \times K_D$$

C_S = sample concentration [µg/ml]
A_S = sample signal [µV x s]
C_R = mean reference concentration [µg/ml]
A_R = mean reference signal [µV x s]
K_D = dilution factor

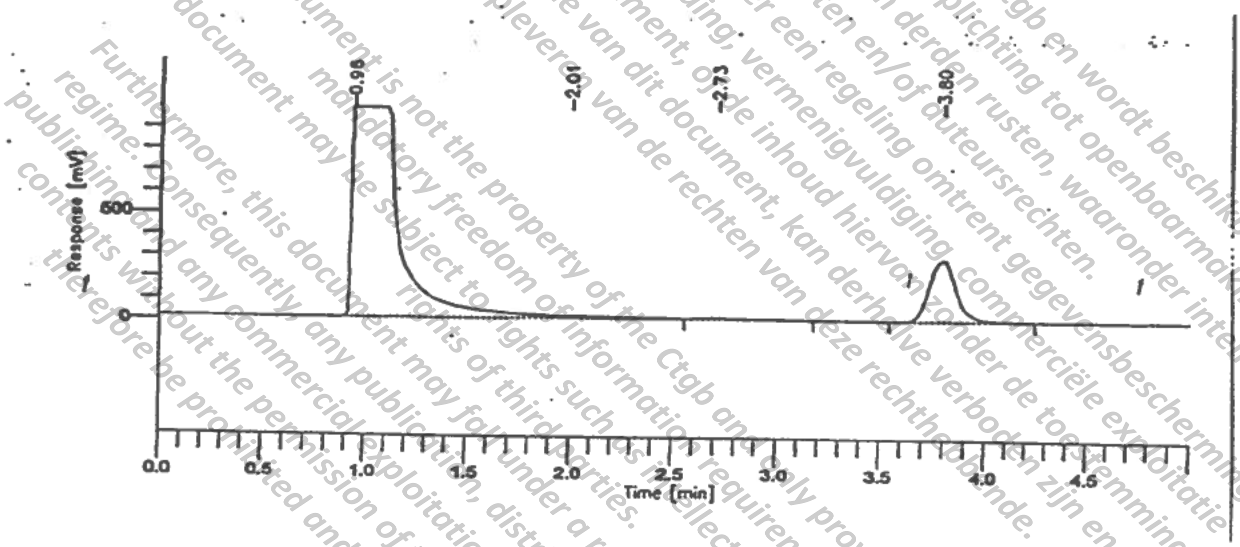
Chromatogram

A typical chromatogram of a reference sample is shown in Figure 1. The appearence of the chromatograms might depend on actual conditions at a particular time (vehicle, solvents, column, environment).

Remarks

Apparatus and parameters are typical examples. They may be changed if required. Any changes must be reported and explained in the raw data. Major changes (principal) must be noted in the report.

Figure 1. Chromatogram of CGA 62826 tech. (Batch: RV-1592/4) in DMSO (50 µg/ml).



Distribution:

Dr. 5.1.2.e Woo (2x)

Mr. [redacted] (1x)