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)	963127 108906 / 6
1.4	Lab. Report N°	963127
1.5	Cross reference to original study / report	5.8.1/10
1.6	Authors	Report: Dr. [redacted] Summary: Dr.
1.7	Date of report	May 23, 1997
1.8	Published / owner	No, Novartis Crop Protection AG
2.1	Testing facility	Genetic Toxicology, Novartis Crop Protection AG, Basel, Switzerland
2.2	Dates of experimental work	November 05 to December 18, 1996
3.	Objectives	To detect a mutagenic activity at the level of gene mutations in bacteria.
4.1	Test substance	CGA 108906 tech. (Metabolite of CGA 48988 and 329351)
4.2	Specification	Batch no. KI-5240/3 / purity 99%
4.3	Storage stability	Reanalysis date: October 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

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 96.3 and 97.3% 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	159.7	20.3	22.3	277.3	30.0	13.3
positive control	1939.7	269.3	1175.0	1842.3	1089.0	171.0
312.50 µg/plate	176.7	20.0	22.0	268.3	29.7	10.3
625.00 µg/plate	154.0	21.3	17.7	266.0	32.3	8.7
1250.00 µg/plate	159.0	21.3	18.3	271.3	30.0	9.3
2500.00 µg/plate	169.7	24.0	17.0	281.0	31.3	8.3
5000.00 µg/plate	144.0	18.7	15.7	271.7	25.3	11.0

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	145.7	13.0	20.0	299.7	20.0	8.7
positive control	1221.7	741.7	413.3	1116.3	308.3	992.0
312.50 µg/plate	139.3	12.3	16.3	283.3	21.0	8.3
625.00 µg/plate	156.0	15.3	16.0	293.7	22.7	9.0
1250.00 µg/plate	146.7	15.0	16.0	287.0	19.7	9.0
2500.00 µg/plate	143.7	20.0	18.3	278.7	20.3	9.3
5000.00 µg/plate	142.3	19.3	16.0	274.3	21.3	8.0

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	141.3	21.3	20.3	292.7	28.3	9.7
positive control	822.7	334.7	621.7	1278.3	577.3	132.3
312.50 µg/plate	146.0	17.3	23.3	293.7	29.0	10.7
625.00 µg/plate	125.7	21.7	16.0	281.3	32.7	13.0
1250.00 µg/plate	138.3	20.0	20.3	282.0	33.3	11.0
2500.00 µg/plate	147.7	18.7	23.3	300.0	30.7	10.0
5000.00 µg/plate	142.3	16.7	22.7	288.7	25.0	11.0

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	142.7	14.0	17.7	285.3	15.3	8.7
positive control	1196.0	748.7	398.0	1187.3	274.0	1002.7
312.50 µg/plate	149.7	18.0	18.3	277.3	18.7	11.3
625.00 µg/plate	157.3	20.3	18.7	275.0	15.7	11.0
1250.00 µg/plate	152.7	18.3	15.3	259.7	22.3	7.7
2500.00 µg/plate	170.3	20.0	16.7	264.0	18.7	7.0
5000.00 µg/plate	142.7	21.7	16.3	268.7	17.3	7.3

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/B.O./August 06, 1997

6

SALMONELLA AND ESCHERICHIA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

Test Number: 963127

Test Substance: CGA 108906 tech.
(Metabolite of CGA 48988)

FINAL REPORT

Author: Dr. 5.1.2.e Woo

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

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

Final Report issued: May 23, 1997

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

Volume 1 of 1 of submission

This report contains 70 numbered pages plus 5 appended pages

- Appendix 1: Personal Records of Staff

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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: Dr. 5.1.2.e Woo

Date: May 23, 1997

For Facility Management: Dr. 5.1.2.e Woo

Date: May 23, 1997

For the Sponsor:

5.1.2.e Woo

Date: May 29, 1997

PAGE RESERVED FOR FLAGGING STATEMENTS

SIGNATURES

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

Study Director: Dr. 5.1.2.e Woo

Date: May 23, 1997

For Facility Management: Dr. 5.1.2.e Woo

Date: May 23, 1997

Quality Assurance Statement

Novartis Crop Protection AG, GLP Quality Assurance, R & D Services, CH-4002 Basel
(Successor in business of Ciba-Geigy Ltd. and Sandoz Ltd.)

Study	963127
Test Substance	CGA 108906 tech.
Study Title	Salmonella and Escherichia/Mammalian-Microsome Mutagenicity Test
Study Director	Dr. [redacted]
QA-Inspector	[redacted]

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

Activity	Performed	Reported
Facility Based Inspection	September 19, 1996	September 20, 1996
Facility Based Inspection	October 24, 1996	October 25, 1996
Protocol Audit	October 25, 1996	October 25, 1996
Process Based Inspection	December 10, 1996	December 10, 1996
Final Report Audit	May 22, 1997	May 22, 1997

May 23, 1997
Date

[redacted]
Inspector Quality Assurance

Form: QSSTAT12

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COMPOUND INFORMATION

Test material:	CGA 108906 tech. (Metabolite of CGA 48988)
Batch No.:	KI-5240/3
Purity:	99%
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:	October 31, 1999
Storage conditions:	0 to 10°C
Material submitted by:	Novartis Crop Protection AG Basel, Switzerland

GENERAL

Type of study:	Salmonella and Escherichia/Mammalian-Microsome Mutagenicity Test. According to SOP No. 30 50 03, Novartis Crop Protection AG, Basel, 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 AG Basel, Switzerland Toxicology, Genetic Toxicology
Archives:	Toxicology, Archives of Genetic Toxicology Novartis Crop Protection AG Basel, Switzerland
Personnel:	Technical conduct: Mr. [redacted] Mrs [redacted]
Study Director:	Dr. [redacted]

Principal Investigator*: (responsible for analytical study)	Mrs. 5.1.2.e Woo Novartis Crop Protection AG Basel, Switzerland Toxicology Laboratories of Cell Biology
Sponsor:	Novartis Crop Protection AG Basel, Switzerland
Sponsor monitoring scientist:	Dr. 5.1.2.e Woo
Test number:	963127
Vehicle:	Dimethylsulfoxide
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:	October 24, 1996
Experimental start date:	November 05, 1996
Experimental termination date:	December 18, 1996
Study termination date:	May 23, 1997

*) Since January 01, 1997, the testing facilities Genetic Toxicology and Cell Biology are parts of the new testing facility Toxicology. Accordingly, the term Principal Investigator changed to Collaborating Scientist.

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-named companies. This applies also to all aspects concerned with the requirements of Good Laboratory Practice.

ABSTRACT

CGA 108906 tech. (Metabolite of CGA 48988), identified as solid, purity 99%, batch no. KI-5240/3, 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 the same concentrations. 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 108906 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 108906 tech. 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. [redacted], Berkeley, USA (1983). Strain TA 100 was obtained from [redacted] Hoffmann-La Roche Limited, Basel, Switzerland (1986). Strain TA 1537 was obtained from [redacted], 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
Dimethylsulfoxide	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 Novartis Crop Protection AG, 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 108906 tech. was dissolved in DMSO at room temperature at 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 (experiments with activation) 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 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 in N separate experiments reported in the period of January 01, 1996 to December 31, 1996 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		129.28	27.03	66	70-220
	2-Aminoanthracene	1.5	1645.98	313.04	66	
TA 1535	Negative control		15.72	3.99	66	7-35
	Cyclophosphamide	200.0	248.21	83.25	66	
WP2 uvrA	Negative control		25.35	5.66	66	8-50
	2-Aminoanthracene	20.0	842.93	152.30	66	
TA102	Negative control		271.64	42.14	66	150-360
	2-Aminoanthracene	5.0	1505.93	306.65	66	
TA 98	Negative control		39.09	8.74	66	20-70
	2-Aminoanthracene	1.5	1669.37	311.87	66	
TA 1537	Negative control		12.34	4.14	66	5-30
	2-Aminoanthracene	1.5	285.34	79.27	66	

With metabolic activation, preincubation assay						
Strain	**Substance**	**µg/plate**	**Mean**	**SD**	**N**	**Acceptable range**
TA 100	Negative control		129.84	20.55	6	70-220
	2-Aminoanthracene	1.5	1543.46	305.46	6	
TA 1535	Negative control		13.56	3.82	6	7-35
	Cyclophosphamide	200.0	255.39	34.22	6	
WP2 uvrA	Negative control		24.00	4.87	6	8-50
	2-Aminoanthracene	20.0	686.22	135.84	6	
TA102	Negative control		268.67	31.15	6	150-360
	2-Aminoanthracene	5.0	1350.50	381.38	6	
TA 98	Negative control		34.89	10.09	6	20-70
	2-Aminoanthracene	1.5	1470.89	201.80	6	
TA 1537	Negative control		11.89	3.34	6	5-30
	2-Aminoanthracene	1.5	215.06	72.44	6	

Without metabolic activation, standard plate incorporation assay						
Strain	**Substance**	**µg/plate**	**Mean**	**SD**	**N**	**Acceptable range**
TA 100	Negative control		121.00	20.52	72	70-220
	Sodium azide	2.0	948.53	145.86	72	
TA 1535	Negative control		15.82	3.61	72	7-30
	Sodium azide	2.0	742.54	95.77	72	
WP2 uvrA	Negative control		22.44	5.54	72	8-40
	4-Nitroquinoline	2.0	815.09	189.37	72	
TA 102	Negative control		248.77	42.01	72	150-360
	Mitomycin-C	0.5	1074.33	192.21	72	
TA 98	Negative control		26.54	7.48	72	12-50
	2-Nitrofluorene	5.0	790.15	172.51	72	
TA 1537	Negative control		10.02	2.86	72	3-20
	9-Aminoacridine	80.0	1015.52	219.23	72	

RESULTS

Range finding test

Six concentrations of CGA 108906 tech. (Metabolite of CGA 48988) 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 108906 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 108906 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. No precipitates or aggregates of the test material were observed on the agar plates.

Analytical control (see Report of Analytical Determinations)

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 with UV detection. The values found by analysis of the different samples were in agreement with the nominal concentrations, 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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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 : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 100

Treatment	Colony counts	Mean
Negative control	126	
CGA 108906 tech.:		
20.58 µg/plate	122	
61.73 µg/plate	124	
185.19 µg/plate	97	
555.56 µg/plate	98	
1666.67 µg/plate	80	
5000.00 µg/plate	64	

Treatment	Remarks	Factor
Negative control	-	1.00
CGA 108906 tech.:		
20.58 µg/plate	-	0.97
61.73 µg/plate	-	0.98
185.19 µg/plate	-	0.77
555.56 µg/plate	-	0.78
1666.67 µg/plate	-	0.63
5000.00 µg/plate	-	0.51

TABLE 2 : RANGE FINDING TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 100

Treatment	Colony counts	Mean
Negative control	101	
CGA 108906 tech.:		
20.58 µg/plate	79	
61.73 µg/plate	110	
185.19 µg/plate	105	
555.56 µg/plate	85	
1666.67 µg/plate	93	
5000.00 µg/plate	64	

Treatment	Remarks	Factor
Negative control	-	1.00
CGA 108906 tech.:		
20.58 µg/plate	-	0.78
61.73 µg/plate	-	1.09
185.19 µg/plate	-	1.04
555.56 µg/plate	-	0.84
1666.67 µg/plate	-	0.92
5000.00 µg/plate	-	0.63

TABLE 3 : RANGE FINDING TEST
Experiment with metabolic activation

Test number	:	**963127**
Experiment	:	**Original**
Test substance	:	**CGA 108906 tech.**
Batch	:	**KI-5240/3**
Strain	:	**WP2 uvrA**

Treatment	Colony counts	Mean
Negative control	14	
CGA 108906 tech.:		
20.58 µg/plate	8	
61.73 µg/plate	13	
185.19 µg/plate	12	
555.56 µg/plate	9	
1666.67 µg/plate	8	
5000.00 µg/plate	8	

Treatment	Remarks	Factor
Negative control	-	1.00
CGA 108906 tech.:		
20.58 µg/plate	-	0.57
61.73 µg/plate	-	0.93
185.19 µg/plate	-	0.86
555.56 µg/plate	-	0.64
1666.67 µg/plate	-	0.57
5000.00 µg/plate	-	0.57

TABLE 4 : RANGE FINDING TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : WP2 uvrA

Treatment	Colony counts	Mean
Negative control	12	
CGA 108906 tech.:		
20.58 µg/plate	15	
61.73 µg/plate	12	
185.19 µg/plate	16	
555.56 µg/plate	8	
1666.67 µg/plate	8	
5000.00 µg/plate	14	

Treatment	Remarks	Factor
Negative control	-	1.00
CGA 108906 tech.:		
20.58 µg/plate	-	1.25
61.73 µg/plate	-	1.00
185.19 µg/plate	-	1.33
555.56 µg/plate	-	0.67
1666.67 µg/plate	-	0.67
5000.00 µg/plate	-	1.17

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

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	159.67	TA 1535	Negative control	20.33
	312.50 µg/plate	176.67		312.50 µg/plate	20.00
	625.00 µg/plate	154.00		625.00 µg/plate	21.33
	1250.00 µg/plate	159.00		1250.00 µg/plate	21.33
	2500.00 µg/plate	169.67		2500.00 µg/plate	24.00
	5000.00 µg/plate	144.00		5000.00 µg/plate	18.67
	Positive control	1939.67		Positive control	269.33
WP2 uvrA	Negative control	22.33	TA 98	Negative control	30.00
	312.50 µg/plate	22.00		312.50 µg/plate	29.67
	625.00 µg/plate	17.67		625.00 µg/plate	32.33
	1250.00 µg/plate	18.33		1250.00 µg/plate	30.00
	2500.00 µg/plate	17.00		2500.00 µg/plate	31.33
	5000.00 µg/plate	15.67		5000.00 µg/plate	25.33
	Positive control	1175.00		Positive control	1089.00
TA 1537	Negative control	13.33	TA 102	Negative control	277.33
	312.50 µg/plate	10.33		312.50 µg/plate	268.33
	625.00 µg/plate	8.67		625.00 µg/plate	266.00
	1250.00 µg/plate	9.33		1250.00 µg/plate	271.33
	2500.00 µg/plate	8.33		2500.00 µg/plate	281.00
	5000.00 µg/plate	11.00		5000.00 µg/plate	271.67
	Positive control	171.00		Positive control	1842.33

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

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	145.67	TA 1535	Negative control	13.00
	312.50 µg/plate	139.33		312.50 µg/plate	12.33
	625.00 µg/plate	156.00		625.00 µg/plate	15.33
	1250.00 µg/plate	146.67		1250.00 µg/plate	15.00
	2500.00 µg/plate	143.67		2500.00 µg/plate	20.00
	5000.00 µg/plate	142.33		5000.00 µg/plate	19.33
	Positive control	1221.67		Positive control	741.67
WP2 uvrA	Negative control	20.00	TA 98	Negative control	20.00
	312.50 µg/plate	16.33		312.50 µg/plate	21.00
	625.00 µg/plate	16.00		625.00 µg/plate	22.67
	1250.00 µg/plate	16.00		1250.00 µg/plate	19.67
	2500.00 µg/plate	18.33		2500.00 µg/plate	20.33
	5000.00 µg/plate	16.00		5000.00 µg/plate	21.33
	Positive control	413.33		Positive control	308.33
TA 1537	Negative control	8.67	TA 102	Negative control	299.67
	312.50 µg/plate	8.33		312.50 µg/plate	283.33
	625.00 µg/plate	9.00		625.00 µg/plate	293.67
	1250.00 µg/plate	9.00		1250.00 µg/plate	287.00
	2500.00 µg/plate	9.33		2500.00 µg/plate	278.67
	5000.00 µg/plate	8.00		5000.00 µg/plate	274.33
	Positive control	992.00		Positive control	1116.33

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

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	141.33	TA 1535	Negative control	21.33
	312.50 µg/plate	146.00		312.50 µg/plate	17.33
	625.00 µg/plate	125.67		625.00 µg/plate	21.67
	1250.00 µg/plate	138.33		1250.00 µg/plate	20.00
	2500.00 µg/plate	147.67		2500.00 µg/plate	18.67
	5000.00 µg/plate	142.33		5000.00 µg/plate	16.67
	Positive control	822.67		Positive control	334.67
WP2 uvrA	Negative control	20.33	TA 98	Negative control	28.33
	312.50 µg/plate	23.33		312.50 µg/plate	29.00
	625.00 µg/plate	16.00		625.00 µg/plate	32.67
	1250.00 µg/plate	20.33		1250.00 µg/plate	33.33
	2500.00 µg/plate	23.33		2500.00 µg/plate	30.67
	5000.00 µg/plate	22.67		5000.00 µg/plate	25.00
	Positive control	621.67		Positive control	577.33
TA 1537	Negative control	9.67	TA 102	Negative control	292.67
	312.50 µg/plate	10.67		312.50 µg/plate	293.67
	625.00 µg/plate	13.00		625.00 µg/plate	281.33
	1250.00 µg/plate	11.00		1250.00 µg/plate	282.00
	2500.00 µg/plate	10.00		2500.00 µg/plate	300.00
	5000.00 µg/plate	11.00		5000.00 µg/plate	288.67
	Positive control	132.33		Positive control	1278.33

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

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	142.67	TA 1535	Negative control	14.00
	312.50 µg/plate	149.67		312.50 µg/plate	18.00
	625.00 µg/plate	157.33		625.00 µg/plate	20.33
	1250.00 µg/plate	152.67		1250.00 µg/plate	18.33
	2500.00 µg/plate	170.33		2500.00 µg/plate	20.00
	5000.00 µg/plate	142.67		5000.00 µg/plate	21.67
	Positive control	1196.00		Positive control	748.67
WP2 uvrA	Negative control	17.67	TA 98	Negative control	15.33
	312.50 µg/plate	18.33		312.50 µg/plate	18.67
	625.00 µg/plate	18.67		625.00 µg/plate	15.67
	1250.00 µg/plate	15.33		1250.00 µg/plate	22.33
	2500.00 µg/plate	16.67		2500.00 µg/plate	18.67
	5000.00 µg/plate	16.33		5000.00 µg/plate	17.33
	Positive control	398.00		Positive control	274.00
TA 1537	Negative control	8.67	TA 102	Negative control	285.33
	312.50 µg/plate	11.33		312.50 µg/plate	277.33
	625.00 µg/plate	11.00		625.00 µg/plate	275.00
	1250.00 µg/plate	7.67		1250.00 µg/plate	259.67
	2500.00 µg/plate	7.00		2500.00 µg/plate	264.00
	5000.00 µg/plate	7.33		5000.00 µg/plate	268.67
	Positive control	1002.67		Positive control	1187.33

TABLE 9 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 100

Treatment	Colony counts			Mean
Negative control	157	145	177	159.67
CGA 108906 tech.:				
312.50 µg/plate	189	169	172	176.67
625.00 µg/plate	165	153	144	154.00
1250.00 µg/plate	162	147	168	159.00
2500.00 µg/plate	185	172	152	169.67
5000.00 µg/plate	149	137	146	144.00
2-Aminoanthracene 1.50 µg/plate	1885	2022	1912	1939.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	1.11
625.00 µg/plate	-	-	-	0.96
1250.00 µg/plate	-	-	-	1.00
2500.00 µg/plate	-	-	-	1.06
5000.00 µg/plate	-	-	-	0.90
2-Aminoanthracene	-	-	-	12.15

TABLE 10 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 1535

Treatment	Colony counts			Mean
Negative control	16	19	26	20.33
CGA 108906 tech.:				
312.50 µg/plate	23	15	22	20.00
625.00 µg/plate	20	22	22	21.33
1250.00 µg/plate	20	19	25	21.33
2500.00 µg/plate	23	24	25	24.00
5000.00 µg/plate	23	15	18	18.67
Cyclophosphamide 200.00 µg/plate	253	287	268	269.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	0.98
625.00 µg/plate	-	-	-	1.05
1250.00 µg/plate	-	-	-	1.05
2500.00 µg/plate	-	-	-	1.18
5000.00 µg/plate	-	-	-	0.92
Cyclophosphamide	-	-	-	13.25

TABLE 11 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : WP2 uvrA

Treatment	Colony counts			Mean
Negative control	21	23	23	22.33
CGA 108906 tech.:				
312.50 µg/plate	26	21	19	22.00
625.00 µg/plate	19	15	19	17.67
1250.00 µg/plate	19	17	19	18.33
2500.00 µg/plate	23	16	12	17.00
5000.00 µg/plate	13	18	16	15.67
2-Aminoanthracene 20.00 µg/plate	1276	1129	1120	1175.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	0.99
625.00 µg/plate	-	-	-	0.79
1250.00 µg/plate	-	-	-	0.82
2500.00 µg/plate	-	-	-	0.76
5000.00 µg/plate	-	-	-	0.70
2-Aminoanthracene	-	-	-	52.61

TABLE 12 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 98

Treatment	Colony counts			Mean
Negative control	27	25	38	30.00
CGA 108906 tech.:				
312.50 µg/plate	33	24	32	29.67
625.00 µg/plate	33	33	31	32.33
1250.00 µg/plate	33	32	25	30.00
2500.00 µg/plate	32	26	36	31.33
5000.00 µg/plate	25	27	24	25.33
2-Aminoanthracene 1.50 µg/plate	1047	1209	1011	1089.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	0.99
625.00 µg/plate	-	-	-	1.08
1250.00 µg/plate	-	-	-	1.00
2500.00 µg/plate	-	-	-	1.04
5000.00 µg/plate	-	-	-	0.84
2-Aminoanthracene	-	-	-	36.30

TABLE 13 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 1537

Treatment	Colony counts			Mean
Negative control	12	11	17	13.33
CGA 108906 tech.:				
312.50 µg/plate	11	14	6	10.33
625.00 µg/plate	8	9	9	8.67
1250.00 µg/plate	7	13	8	9.33
2500.00 µg/plate	5	9	11	8.33
5000.00 µg/plate	14	13	6	11.00
2-Aminoanthracene 1.50 µg/plate	158	171	184	171.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	0.78
625.00 µg/plate	-	-	-	0.65
1250.00 µg/plate	-	-	-	0.70
2500.00 µg/plate	-	-	-	0.63
5000.00 µg/plate	-	-	-	0.83
2-Aminoanthracene	-	-	-	12.83

TABLE 14 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 102

Treatment	Colony counts			Mean
Negative control	279	282	271	277.33
CGA 108906 tech.:				
312.50 µg/plate	270	258	277	268.33
625.00 µg/plate	273	264	261	266.00
1250.00 µg/plate	273	258	283	271.33
2500.00 µg/plate	276	289	278	281.00
5000.00 µg/plate	281	281	253	271.67
2-Aminoanthracene 5.00 µg/plate	1854	1840	1833	1842.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	0.97
625.00 µg/plate	-	-	-	0.96
1250.00 µg/plate	-	-	-	0.98
2500.00 µg/plate	-	-	-	1.01
5000.00 µg/plate	-	-	-	0.98
2-Aminoanthracene	-	-	-	6.64

TABLE 15 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 100

Treatment	Colony counts			Mean
Negative control	137	148	152	145.67
CGA 108906 tech.:				
312.50 µg/plate	124	159	135	139.33
625.00 µg/plate	165	153	150	156.00
1250.00 µg/plate	127	156	157	146.67
2500.00 µg/plate	134	156	141	143.67
5000.00 µg/plate	145	149	133	142.33
Sodium azide 2.00 µg/plate	1224	1232	1209	1221.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	0.96
625.00 µg/plate	-	-	-	1.07
1250.00 µg/plate	-	-	-	1.01
2500.00 µg/plate	-	-	-	0.99
5000.00 µg/plate	-	-	-	0.98
Sodium azide	-	-	-	8.39

TABLE 16 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 1535

Treatment	Colony counts			Mean
Negative control	16	8	15	13.00
CGA 108906 tech.:				
312.50 µg/plate	14	11	12	12.33
625.00 µg/plate	17	15	14	15.33
1250.00 µg/plate	18	16	11	15.00
2500.00 µg/plate	20	22	18	20.00
5000.00 µg/plate	25	20	13	19.33
Sodium azide 2.00 µg/plate	729	776	720	741.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	0.95
625.00 µg/plate	-	-	-	1.18
1250.00 µg/plate	-	-	-	1.15
2500.00 µg/plate	-	-	-	1.54
5000.00 µg/plate	-	-	-	1.49
Sodium azide	-	-	-	57.05

TABLE 17 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : WP2 uvrA

Treatment	Colony counts			Mean
Negative control	14	24	22	20.00
CGA 108906 tech.:				
312.50 µg/plate	18	19	12	16.33
625.00 µg/plate	24	13	11	16.00
1250.00 µg/plate	15	18	15	16.00
2500.00 µg/plate	18	15	22	18.33
5000.00 µg/plate	19	15	14	16.00
4-NQO 2.00 µg/plate	428	398	414	413.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	0.82
625.00 µg/plate	-	-	-	0.80
1250.00 µg/plate	-	-	-	0.80
2500.00 µg/plate	-	-	-	0.92
5000.00 µg/plate	-	-	-	0.80
4-NQO	-	-	-	20.67

TABLE 18 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number	:	**963127**
Experiment	:	**Original**
Test substance	:	**CGA 108906 tech.**
Batch	:	**KI-5240/3**
Strain	:	**TA 98**

Treatment	Colony counts			Mean
Negative control	20	19	21	20.00
CGA 108906 tech.:				
312.50 μg/plate	22	25	16	21.00
625.00 μg/plate	27	21	20	22.67
1250.00 μg/plate	18	15	26	19.67
2500.00 μg/plate	19	21	21	20.33
5000.00 μg/plate	21	24	19	21.33
2-Nitrofluorene 5.00 μg/plate	321	295	309	308.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 μg/plate	-	-	-	1.05
625.00 μg/plate	-	-	-	1.13
1250.00 μg/plate	-	-	-	0.98
2500.00 μg/plate	-	-	-	1.02
5000.00 μg/plate	-	-	-	1.07
2-Nitrofluorene	-	-	-	15.42

TABLE 19 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 1537

Treatment	Colony counts			Mean
Negative control	10	9	7	8.67
CGA 108906 tech.:				
312.50 µg/plate	9	9	7	8.33
625.00 µg/plate	8	11	8	9.00
1250.00 µg/plate	10	6	11	9.00
2500.00 µg/plate	6	11	11	9.33
5000.00 µg/plate	10	4	10	8.00
9-Aminoacridine 80.00 µg/plate	962	1011	1003	992.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	0.96
625.00 µg/plate	-	-	-	1.04
1250.00 µg/plate	-	-	-	1.04
2500.00 µg/plate	-	-	-	1.08
5000.00 µg/plate	-	-	-	0.92
9-Aminoacridine	-	-	-	114.46

TABLE 20 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Original
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 102

Treatment	Colony counts			Mean
Negative control	308	314	277	299.67
CGA 108906 tech.:				
312.50 µg/plate	303	269	278	283.33
625.00 µg/plate	297	281	303	293.67
1250.00 µg/plate	295	296	270	287.00
2500.00 µg/plate	300	266	270	278.67
5000.00 µg/plate	276	282	265	274.33
Mitomycin-C 0.50 µg/plate	1153	1131	1065	1116.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	0.95
625.00 µg/plate	-	-	-	0.98
1250.00 µg/plate	-	-	-	0.96
2500.00 µg/plate	-	-	-	0.93
5000.00 µg/plate	-	-	-	0.92
Mitomycin-C	-	-	-	3.73

TABLE 21 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number	:	**963127**
Experiment	:	**Confirmatory**
Test substance	:	**CGA 108906 tech.**
Batch	:	**KI-5240/3**
Strain	:	**TA 100**

Treatment	**Colony counts**			**Mean**
Negative control	141	112	171	141.33
CGA 108906 tech.:				
312.50 µg/plate	156	133	149	146.00
625.00 µg/plate	124	128	125	125.67
1250.00 µg/plate	141	135	139	138.33
2500.00 µg/plate	144	153	146	147.67
5000.00 µg/plate	139	132	156	142.33
2-Aminoanthracene 1.50 µg/plate	999	728	741	822.67

Treatment	**Remarks**			**Factor**
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	1.03
625.00 µg/plate	-	-	-	0.89
1250.00 µg/plate	-	•	-	0.98
2500.00 µg/plate	-	-	-	1.04
5000.00 µg/plate	-	-	-	1.01
2-Aminoanthracene	-	-	-	5.82

TABLE 22 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 1535

Treatment	Colony counts			Mean
Negative control	23	21	20	21.33
CGA 108906 tech.:				
312.50 µg/plate	14	21	17	17.33
625.00 µg/plate	23	20	22	21.67
1250.00 µg/plate	23	15	22	20.00
2500.00 µg/plate	20	17	19	18.67
5000.00 µg/plate	24	10	16	16.67
Cyclophosphamide 200.00 µg/plate	336	316	352	334.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	0.81
625.00 µg/plate	-	-	-	1.02
1250.00 µg/plate	-	-	-	0.94
2500.00 µg/plate	-	-	-	0.87
5000.00 µg/plate	-	-	-	0.78
Cyclophosphamide	-	-	-	15.69

TABLE 23 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : WP2 uvrA

Treatment	Colony counts			Mean
Negative control	20	25	16	20.33
CGA 108906 tech.:				
312.50 µg/plate	22	26	22	23.33
625.00 µg/plate	15	18	15	16.00
1250.00 µg/plate	19	22	20	20.33
2500.00 µg/plate	28	19	23	23.33
5000.00 µg/plate	19	25	24	22.67
2-Aminoanthracene 20.00 µg/plate	530	541	794	621.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	1.15
625.00 µg/plate	-	-	-	0.79
1250.00 µg/plate	-	-	-	1.00
2500.00 µg/plate	-	-	-	1.15
5000.00 µg/plate	-	-	-	1.11
2-Aminoanthracene	-	-	-	30.57

TABLE 24 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 98

Treatment	Colony counts			Mean
Negative control	32	32	21	28.33
CGA 108906 tech.:				
312.50 µg/plate	28	26	33	29.00
625.00 µg/plate	29	30	39	32.67
1250.00 µg/plate	40	32	28	33.33
2500.00 µg/plate	29	31	32	30.67
5000.00 µg/plate	25	18	32	25.00
2-Aminoanthracene 1.50 µg/plate	544	489	699	577.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	1.02
625.00 µg/plate	-	-	-	1.15
1250.00 µg/plate	-	-	-	1.18
2500.00 µg/plate	-	-	-	1.08
5000.00 µg/plate	-	-	-	0.88
2-Aminoanthracene	-	-	-	20.38

TABLE 25 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 1537

Treatment	Colony counts			Mean
Negative control	9	9	11	9.67
CGA 108906 tech.:				
312.50 µg/plate	9	11	12	10.67
625.00 µg/plate	14	12	13	13.00
1250.00 µg/plate	9	8	16	11.00
2500.00 µg/plate	10	11	9	10.00
5000.00 µg/plate	6	15	12	11.00
2-Aminoanthracene 1.50 µg/plate	141	121	135	132.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	1.10
625.00 µg/plate	-	-	-	1.34
1250.00 µg/plate	-	-	-	1.14
2500.00 µg/plate	-	-	-	1.03
5000.00 µg/plate	-	-	-	1.14
2-Aminoanthracene	-	-	-	13.69

TABLE 26 : MUTAGENICITY TEST
Experiment with metabolic activation

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 102

Treatment	Colony counts			Mean
Negative control	290	282	306	292.67
CGA 108906 tech.:				
312.50 µg/plate	293	285	303	293.67
625.00 µg/plate	279	294	271	281.33
1250.00 µg/plate	273	289	284	282.00
2500.00 µg/plate	281	307	312	300.00
5000.00 µg/plate	284	297	285	288.67
2-Aminoanthracene 4.00 µg/plate	1012	1389	1434	1278.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	1.00
625.00 µg/plate	-	-	-	0.96
1250.00 µg/plate	-	-	-	0.96
2500.00 µg/plate	-	-	-	1.03
5000.00 µg/plate	-	-	-	0.99
2-Aminoanthracene	-	-	-	4.37

TABLE 27 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 100

Treatment	Colony counts			Mean
Negative control	149	132	147	142.67
CGA 108906 tech.:				
312.50 µg/plate	164	138	147	149.67
625.00 µg/plate	160	173	139	157.33
1250.00 µg/plate	132	164	162	152.67
2500.00 µg/plate	182	160	169	170.33
5000.00 µg/plate	153	141	134	142.67
Sodium azide 2.00 µg/plate	1231	1229	1128	1196.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	1.05
625.00 µg/plate	-	-	-	1.10
1250.00 µg/plate	-	-	-	1.07
2500.00 µg/plate	-	-	-	1.19
5000.00 µg/plate	-	-	-	1.00
Sodium azide	-	-	-	8.38

TABLE 28 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 1535

Treatment	Colony counts			Mean
Negative control	12	16	14	14.00
CGA 108906 tech.:				
312.50 µg/plate	17	23	14	18.00
625.00 µg/plate	21	15	25	20.33
1250.00 µg/plate	20	20	15	18.33
2500.00 µg/plate	21	24	15	20.00
5000.00 µg/plate	22	19	24	21.67
Sodium azide 2.00 µg/plate	785	709	752	748.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	1.29
625.00 µg/plate	-	-	-	1.45
1250.00 µg/plate	-	-	-	1.31
2500.00 µg/plate	-	-	-	1.43
5000.00 µg/plate	-	-	-	1.55
Sodium azide	-	-	-	53.48

TABLE 29 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : WP2 uvrA

Treatment	Colony counts			Mean
Negative control	15	19	19	17.67
CGA 108906 tech.:				
312.50 µg/plate	15	24	16	18.33
625.00 µg/plate	18	23	15	18.67
1250.00 µg/plate	13	16	17	15.33
2500.00 µg/plate	14	18	18	16.67
5000.00 µg/plate	14	13	22	16.33
4-NQO 2.00 µg/plate	379	413	402	398.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	1.04
625.00 µg/plate	-	-	-	1.06
1250.00 µg/plate	-	-	-	0.87
2500.00 µg/plate	-	-	-	0.94
5000.00 µg/plate	-	-	-	0.92
4-NQO	-	-	-	22.53

TABLE 30 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 98

Treatment	Colony counts			Mean
Negative control	14	15	17	15.33
CGA 108906 tech.:				
312.50 µg/plate	16	22	18	18.67
625.00 µg/plate	18	12	17	15.67
1250.00 µg/plate	19	26	22	22.33
2500.00 µg/plate	23	15	18	18.67
5000.00 µg/plate	21	14	17	17.33
2-Nitrofluorene 5.00 µg/plate	258	287	277	274.00

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	1.22
625.00 µg/plate	-	-	-	1.02
1250.00 µg/plate	-	-	-	1.46
2500.00 µg/plate	-	-	-	1.22
5000.00 µg/plate	-	-	-	1.13
2-Nitrofluorene	-	-	-	17.87

TABLE 31 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 1537

Treatment	Colony counts			Mean
Negative control	7	11	8	8.67
CGA 108906 tech.:				
312.50 µg/plate	9	11	14	11.33
625.00 µg/plate	8	12	13	11.00
1250.00 µg/plate	5	6	12	7.67
2500.00 µg/plate	10	3	8	7.00
5000.00 µg/plate	7	6	9	7.33
9-Aminoacridine 80.00 µg/plate	1021	978	1009	1002.67

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	1.31
625.00 µg/plate	-	-	-	1.27
1250.00 µg/plate	-	-	-	0.88
2500.00 µg/plate	-	-	-	0.81
5000.00 µg/plate	-	-	-	0.85
9-Aminoacridine	-	-	-	115.69

TABLE 32 : MUTAGENICITY TEST
Experiment without metabolic activation

Test number : 963127
Experiment : Confirmatory
Test substance : CGA 108906 tech.
Batch : KI-5240/3
Strain : TA 102

Treatment	Colony counts			Mean
Negative control	270	292	294	285.33
CGA 108906 tech.:				
312.50 µg/plate	284	280	268	277.33
625.00 µg/plate	271	266	288	275.00
1250.00 µg/plate	277	218	284	259.67
2500.00 µg/plate	253	271	268	264.00
5000.00 µg/plate	273	273	260	268.67
Mitomycin-C 0.50 µg/plate	1255	1202	1105	1187.33

Treatment	Remarks			Factor
Negative control	-	-	-	1.00
CGA 108906 tech.:				
312.50 µg/plate	-	-	-	0.97
625.00 µg/plate	-	-	-	0.96
1250.00 µg/plate	-	-	-	0.91
2500.00 µg/plate	-	-	-	0.93
5000.00 µg/plate	-	-	-	0.94
Mitomycin-C	-	-	-	4.16

SALMONELLA AND ESCHERICHIA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

SUMMARIZED REPORT TO 963127

CGA 108906 tech.

Report of Result of Mutagenicity Test using Microorganisms

1. General Item

Name of the new chemical substance (IUPAC nomenclature)				
Other name	CGA 108906 tech.			
Structural formula or rational formula (or outline of manu-facturing method, in case both are unknown)				
Purity of the new chemical substance tested	99%		Lot of the chemical substance tested	KI-5240/3
Name and concentration of impurities				
CAS number			Vapor pressure	
Molecular weight			Partition coefficient	
Melting point			Appearance at ordinary temperature	solid
Boiling point				
Stability				
Solubility in solvent	Solvent	Solubility	Solvent	Solubility
	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 and November 20, 1996
TA 100	Dr. [redacted] (Hoffmann-La Roche)	June 1986	August 07 and November 20, 1996
TA 102	Prof. [redacted]	January 1983	August 07 and November 20, 1996
TA 1535	Prof. [redacted]	January 1983	August 07 and November 20, 1996
TA 1537	Dr. [redacted] (Hoffmann-La Roche)	July 1994	August 07 and November 20, 1996
E. coli WP2 uvrA	National Collection of Industrial Bacteria, Aberdeen, Scotland	April 1977	August 07 and November 20, 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 (g/kg body weight)	Single application 5 days prior sacrifice 0.5 g/kg in sesame oil
Weight	169 to 186 g		

(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	Lot 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 %
	Dimethylsulfoxide	Merck,Germany	K22294831 5437	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 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	29857033
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	1.79		2.28			
	Main study Orig./Conf.	1.92 / 1.82	1.71 / 1.55	2.73 / 2.70	1.80 / 1.57	1.15 / 1.57	1.65 / 1.00
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 108906 tech.	

12. Others

Testing Institution	**Name**	CIBA-GEIGY Ltd., Genetic Toxicology		
	Address	CH-4002 Basel, Switzerland, Tel.: 41 61/697 11 11		
Administrator	**Name**	Dr.	**Signature**	
			Final education career and specialized field	Dr. phil. II, Zoology University of Basel
Individual Responsible for Archive Storage	**Name**	Mrs.	**Signature**	
Individual Responsible for Quality Assurance	**Name**	Mr.	**Signature**	
Study Director	**Name**	Dr.	**Signature**	
	Years of experience	9 years	**Final education career and specialized field**	Dr. phil. nat., Microbiology University of Frankfurt
Personnel engaged in study	**Name**	Mr *	**Signature**	*
	Years of experience	4 years	**Final education career and specialized field**	See Appendix
Personnel engaged in study	**Name**	Mrs.	**Signature**	
	Years of experience	19 years	**Final education career and specialized field**	See Appendix
Test dates	Study initiation date: October 24, 1996 Experimental start date: November 05, 1996 Experimental termination date: December 18, 1996 Study termination date: May 23, 1997			
Test number	963127			

* has left Genetic Toxicology in February 1997

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 108906 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	157 145 177 (160)	16 19 26 (20)	21 23 23 (22)	279 282 271 (277)	27 25 38 (30)	12 11 17 (13)
	312.50	189 169 172 (177)	23 15 22 (20)	26 21 19 (22)	270 258 277 (268)	33 24 32 (30)	11 14 6 (10)
	625.00	165 153 144 (154)	20 22 22 (21)	19 15 19 (18)	273 264 261 (266)	33 33 31 (32)	8 9 9 (9)
	1250.00	162 147 168 (159)	20 19 25 (21)	19 17 19 (18)	273 258 283 (271)	33 32 25 (30)	7 13 8 (9)
	2500.00	185 172 152 (170)	23 24 25 (24)	23 16 12 (17)	276 289 278 (281)	32 26 36 (31)	5 9 11 (8)
	5000.00	149 137 146 (144)	23 15 18 (19)	13 18 16 (16)	281 281 253 (272)	25 27 24 (25)	14 13 6 (11)
S9 Mix (-)	Solvent control	137 148 152 (146)	16 8 15 (13)	14 24 22 (20)	308 314 277 (300)	20 19 21 (20)	10 9 7 (9)
	312.50	124 159 135 (139)	14 11 12 (12)	18 19 12 (16)	303 269 278 (283)	22 25 16 (21)	9 9 7 (8)
	625.00	165 153 150 (156)	17 15 14 (15)	24 13 11 (16)	297 281 303 (294)	27 21 20 (23)	8 11 8 (9)
	1250.00	127 156 157 (147)	18 16 11 (15)	15 18 15 (16)	295 296 270 (287)	18 15 26 (20)	10 6 11 (9)
	2500.00	134 156 141 (144)	20 22 18 (20)	18 15 22 (18)	300 266 270 (279)	19 21 21 (20)	6 11 11 (9)
	5000.00	145 149 133 (142)	25 20 13 (19)	19 15 14 (16)	276 282 265 (274)	21 24 19 (21)	10 4 10 (8)
Positive control requiring S9 Mix	Name	2-Amino-anthracene	Cyclo-phosphamide	2-Amino-anthracene	2-Amino-anthracene	2-Amino-anthracene	2-Amino-anthracene
	Concentration (µg/plate)	1.50	200.00	20.00	5.00	1.50	1.50
	Number of colonies/plate	1885 2022 1912 (1940)	253 287 268 (269)	1276 1129 1120 (1175)	1854 1840 1833 (1842)	1047 1209 1011 (1089)	158 171 184 (171)
Positive control not requiring S9 Mix	Name	Sodium azide	Sodium azide	4-NQO	Mitomycin-C	2-Nitro-fluorene	9-Amino-acridine
	Concentration (µg/plate)	2.00	2.00	2.00	0.50	5.00	80.00
	Number of colonies/plate	1224 1232 1209 (1222)	729 776 720 (742)	428 398 414 (413)	1153 1131 1065 (1116)	321 295 309 (308)	962 1011 1003 (992)

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 108906 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	141 112 171 (141)	23 21 20 (21)	20 25 16 (20)	290 282 306 (293)	32 32 21 (28)	9 9 11 (10)
	312.50	156 133 149 (146)	14 21 17 (17)	22 26 22 (23)	293 285 303 (294)	28 26 33 (29)	9 11 12 (11)
	625.00	124 128 125 (126)	23 20 22 (22)	15 18 15 (16)	279 294 271 (281)	29 30 39 (33)	14 12 13 (13)
	1250.00	141 135 139 (138)	23 15 22 (20)	19 22 20 (20)	273 289 284 (282)	40 32 28 (33)	9 8 16 (11)
	2500.00	144 153 146 (148)	20 17 19 (19)	28 19 23 (23)	281 307 312 (300)	29 31 32 (31)	10 11 9 (10)
	5000.00	139 132 156 (142)	24 10 16 (17)	19 25 24 (23)	284 297 285 (289)	25 18 32 (25)	6 15 12 (11)
S9 Mix (-)	Solvent control	149 132 147 (143)	12 16 14 (14)	15 19 19 (18)	270 292 294 (285)	14 15 17 (15)	7 11 8 (9)
	312.50	164 138 147 (150)	17 23 14 (18)	15 24 16 (18)	284 280 268 (277)	16 22 18 (19)	9 11 14 (11)
	625.00	160 173 139 (157)	21 15 25 (20)	18 23 15 (19)	271 266 288 (275)	18 12 17 (16)	8 12 13 (11)
	1250.00	132 164 162 (153)	20 20 15 (18)	13 16 17 (15)	277 218 284 (260)	19 26 22 (22)	5 6 12 (8)
	2500.00	182 160 169 (170)	21 24 15 (20)	14 18 18 (17)	253 271 268 (264)	23 15 18 (19)	10 3 8 (7)
	5000.00	153 141 134 (143)	22 19 24 (22)	14 13 22 (16)	273 273 260 (269)	21 14 17 (17)	7 6 9 (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 (μg/plate)	1.50	200.00	20.00	4.00	1.50	1.50
	Number of colonies/plate	999 728 741 (823)	336 316 352 (335)	530 541 794 (622)	1012 1389 1434 (1278)	544 489 699 (577)	141 121 135 (132)
Positive control not requiring S9 Mix	Name	Sodium azide	Sodium azide	4-NQO	Mitomycin-C	2-Nitro-fluorene	9-Amino-acridine
	Concentration (μg/plate)	2.00	2.00	2.00	0.50	5.00	80.00
	Number of colonies/plate	1231 1229 1128 (1196)	785 709 752 (749)	379 413 402 (398)	1255 1202 1105 (1187)	258 287 277 (274)	1021 978 1009 (1003)

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

REPORT OF ANALYTICAL DETERMINATIONS

CIBA-GEIGY Limited

ANALYSIS DATA

Test No. : 963127
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 108906 tech.
Batch No. : KI-5240/3
Vehicle : DMSO
Date of Preparation : November 26, 1996

Purity % : 99 %
Storage conditions : - 20°C

Performed by (Name):

5.1.2.e Woo

5.1.2.e Woo

(Visa)

Study Director:

Dr. 5.1.2.e Woo R-1058.3.62, Tel. : 5.1.2.e Woo

6.12.96 (Date)

5.1.2.e Woo (Signature)

Delivered to Toxicology/Cell Biology: 5.1.2.e Woo

Zellbiologie PP 2.523

Date: 9.12.96 Name: R - 1058.2.50 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	3000.1	96.0
			3018.7	96.6

Analysis method: A 960048

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

18/12/96 (Date)

5.1.2.e Woo (Signature)

Approved by: 5.1.2.e Woo

18.12.96 (Date)

Zellbiologie PP 2.523

(Signature) R - 1058.2.52 5.1.2.e Woo

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

CIBA-GEIGY Limited

ANALYSIS DATA

Test No. : 963127 QA requested : X yes no
Repetition : confirmatory

Test System : SALMONELLA AND ESCHERICHIA/LIVER-MICROSOME TEST

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

Test Substance : CGA 108906 tech.
Batch No. : KI-5240/3 Purity % : 99 %
Vehicle : DMSO Storage conditions : - 20°C
Date of Preparation : December 03, 1996

Performed by (Name): 5.1.2.e Woo

Study Director: Dr. 5.1.2.e Woo, R-1058.3.62, Tel. 5.1.2.e Woo

5.1.2.e Woo (Visa)

6.12.96 (Date) 5.1.2.e Woo (Signature)

Delivered to Toxicology/Cell Biology: 5.1.2.e Woo

Date: 9.12.96 Name: Zellbiologie PP 2.523 R - 1058.2.50 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	3042.3	97.4
			3038.1	97.2

Analysis method: A 960048

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

18/12/96 (Date) 5.1.2.e Woo (Signature)

Approved by: Dr. 5.1.2.e Woo Zellbiologie PP 2.523 R - 1058.2.52 5.1.2.e Woo

17.12.96 (Date) 5.1.2.e Woo (Signature)

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

Determination of CGA 108906 tech. in DMSO

Objective

Determination of the content of CGA 108906 tech. in DMSO by HPLC.

Method Evaluation:	Substance:	CGA 108906 tech.
	Batch:	KI-5240/3
	Matrix:	DMSO

Test Facility

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

Principal Investigator: [redacted] Date/Signature: 17/12/96 [redacted]

Approved: [redacted] Signature: [redacted]

Abstract:

The samples are 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 (230 nm).

Chemicals

DMSO puriss
Acetonitrile (HPLC quality)
o-Phosphoric acid (85% Suprapur)

Test Samples

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

Reference Samples

Two stock solutions of about 15 and 25 mg CGA 108906 tech dissolved in 25 ml DMSO are prepared. These stock solutions are diluted with DMSO to yield final concentrations in the range of the test samples (R1, R2).

HPLC Conditions

Pump:	SP P8800
Detector:	Spectra 100, wavelenth: 230 nm
Integrator:	PE Nelson Turbochrom
Column:	Nucleosil C18, 5 µm, 125x4.6 mm
Mobile phase A:	Acetonitrile
Mobile phase B:	0.05% aqueous phosphoric acid
Gradient:	0 - 10 Min.: 20% - 30 % A
Temperature:	Ambient
Flow rate:	1.5 ml/min
Injection volume:	20 µl
Retention time:	approx. 5 min.
Analysis time:	approx. 10 min.

Injection Sequence

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

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

Calculation

The concentration of CGA 108906 tech in the test sample is calculated from the peak area in comparison with the mean peak area obtained with the reference samples 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
C_R = mean reference concentration [µg/ml]
A_R = mean reference signal
K_D = dilution factor

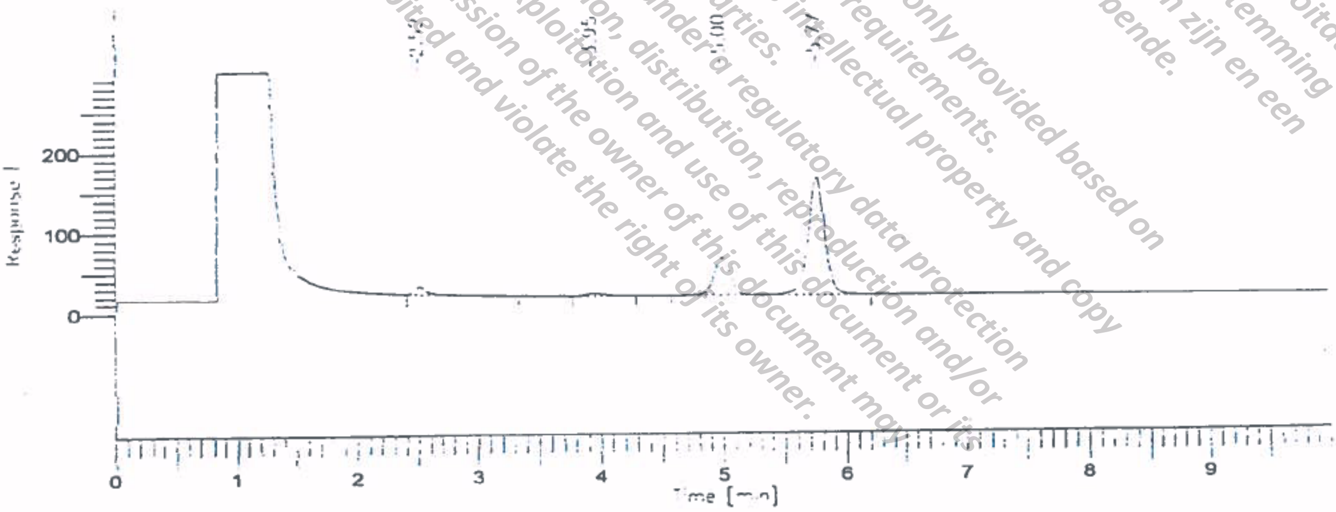
Chromatogram

Typical reference chromatograms of a reference sample are shown in Figure 1 for the detection wavelengths of 230 nm.The appearance of actual 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 108906 tech(Batch: KI-5240/3) in DMSO (31.25 µg/ml) at 230 nm.



SALMONELLA AND ESCHERICHIA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

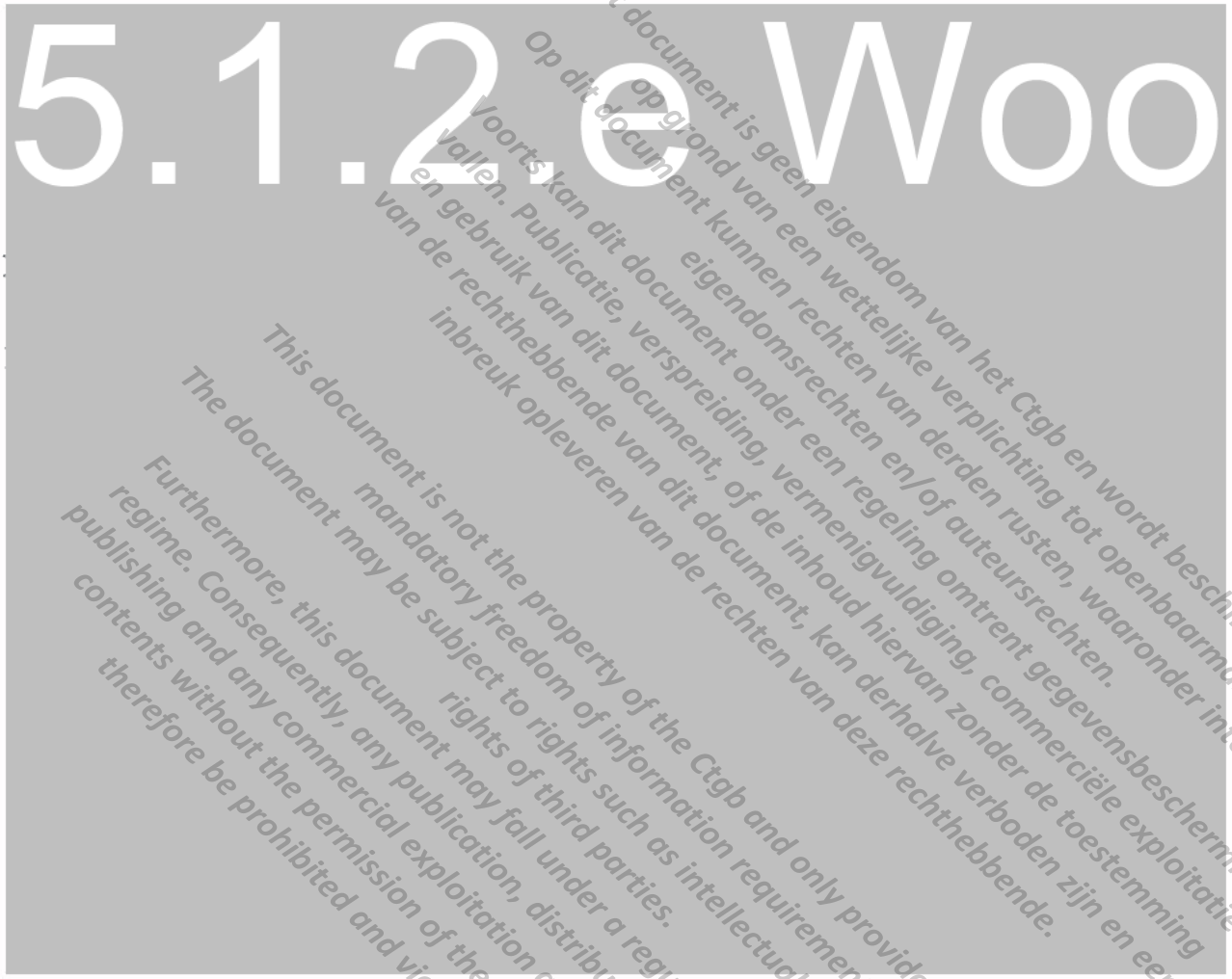
CGA 108906 tech.

APPENDIX 1 TO THE REPORT 963127

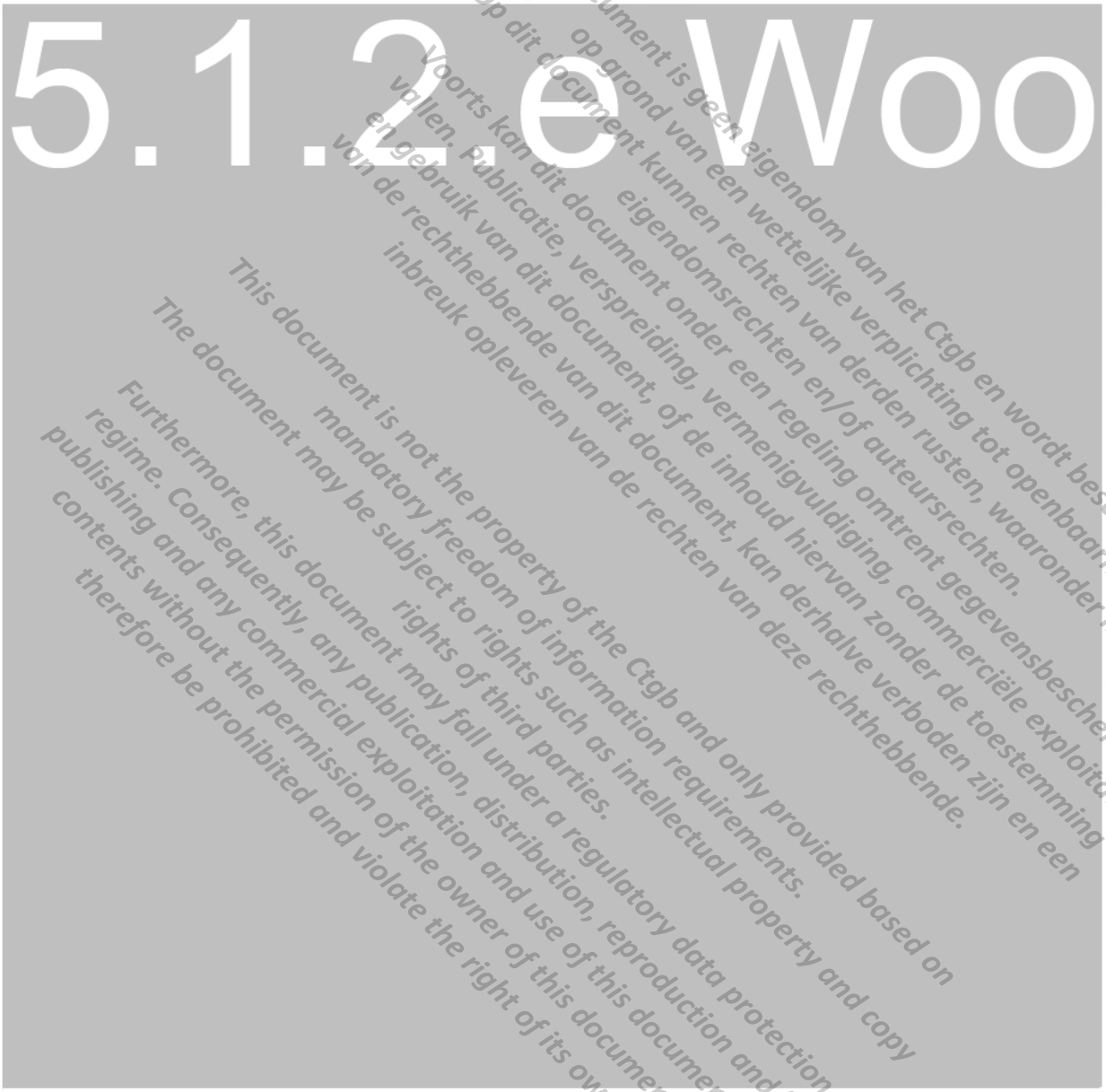
PERSONAL RECORDS OF STAFF

(5 pages)

Appendix to the summarized report

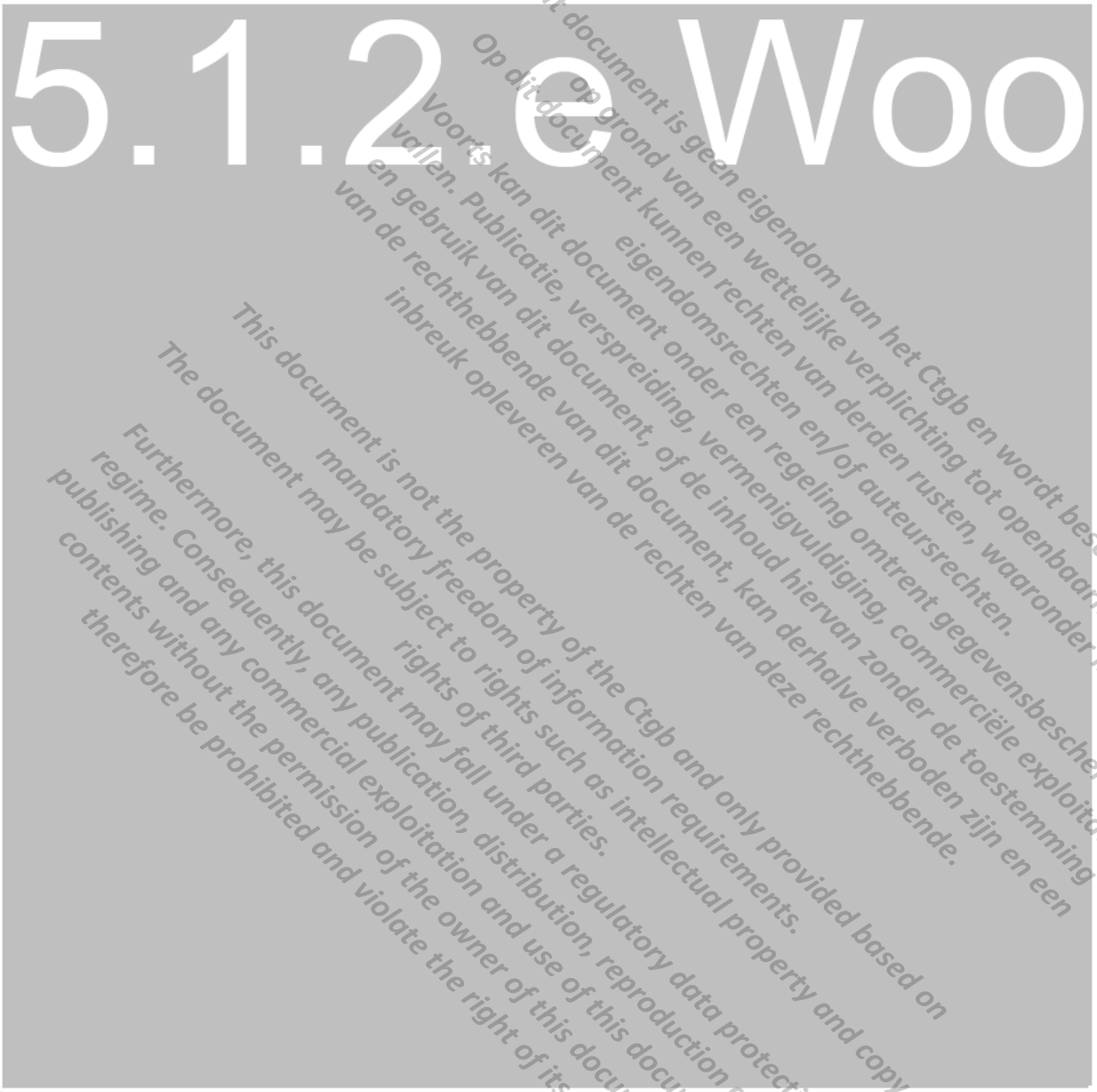


Appendix to the summarized report



Appendix to the summarized report

Appendix to the summarized report



Distribution:

5.1.2.e Woo

(2x)

(1x)