GENE MUTATION TEST WITH CHINESE HAMSTER CELLS V79

Test Number: 983071

Test Substance: CGA 108906 tech. (Metabolite of CGA 329351)

FINAL REPORT

Author: Mr. 5.1.2.e Woo

Testing Facility: Genetic Toxicology
Novartis Crop Protection AG
CH-4002 Basel, Switzerland

Test Guidelines: OECD 476 (1997)
EPA OPPTS 870.5300 (1998)
EEC (1987/88)

Final Report issued: December 03, 1998

Sponsor: Novartis Crop Protection AG
CH-4002 Basel, Switzerland

Volume 1 of 1 of submission

This report contains 67 numbered pages

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GLP COMPLIANCE STATEMENT

This study has been performed in compliance with Good Laboratory Practice (GLP) in Switzerland (Verfahren und Grundsätze der Guten Laborpraxis (GLP) in der Schweiz), Procedures and Principles, March 1986, issued by the Swiss Federal Department of the Interior (Federal Office for Environmental Protection and Federal Office for Public Health) and the Intercantonal Office for the Control of Medicaments. These procedures are consistent with:

United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 160 (FIFRA); Federal Register, August 17, 1989.

Organization for Economic Cooperation and Development Principles of Good Laboratory Practice (Council Decision 81/30, adopted May 12, 1981 and the OECD Recommendation 89/87 concerning the 'Compliance with Principles of Good Laboratory Practice,' adopted October 2, 1989).

Japan Ministry of Agriculture, Forestry and Fisheries, Notification 59 NohSan No. 3850, Agricultural Production Bureau, August 10, 1984.

5.1.2.e Woo

December 03, 1998

Facility Management
Novartis Crop Protection AG

Date

5.1.2.e Woo

December 03, 1998

Mr.
Study Director
Genetic Toxicology
Novartis Crop Protection AG

Date

5.1.2.e Woo

December 17, 1998

Dr. 5.1.2.e Woo
Sponsor
Novartis Crop Protection AG

Date

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SIGNATURES

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

Study Director: Mr. 5.1.2.e Woo

Date: December 03, 1998

Facility Management: Dr. 5.1.2.e Woo

Date: December 03, 1998

Quality Assurance Statement

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

Study	**983071**
Test substance	**CGA 108906 tech.**
Study Title	**Gene Mutation Test with Chinese Hamster Cells V79**
Study Director	5.1.2.e Woo
QA Inspector	

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

Activity	Performed	Reported
Facility Based Inspection	March 26, 1998	March 31, 1998
Protocol Audit	July 9, 1998	July 9, 1998
Facility Based Inspection	August 12, 1998	September 23, 1998
Study Based Inspection	August 24, 1998	August 24, 1998
Final Report Audit	November 27, 1998	November 27, 1998

December 7, 1998
Date

5.1.2.e Woo
Quality Assurance Inspector

Form. QSSTAT01

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COMPOUND INFORMATION

Test substance:	CGA 108906 tech. (Metabolite of CGA 329351)
Batch No:	KI-5240/3
Purity:	99%±2%
Reanalysis date:	October 31, 1999
Stability (of the test compound itself):	Stable (see reanalysis date)
Stability (in the vehicle used, under the conditions of the test):	Stable
Storage conditions:	<10°C
Material submitted by:	Novartis Crop Protection AG CH-4002 Basel, Switzerland

GENERAL

Test No:	983071
Type of study:	Gene mutation test with Chinese Hamster Cells V79. According to SOP No. 30 59 02, Novartis Crop Protection AG, Basel, Switzerland This test is in agreement with • OECD guidelines (1997) [1] • EPA guidelines (1998) [2] • EEC guidelines (1987/88) [3]
Purpose:	Evaluation of any property of the test substance or its metabolites to induce gene mutations
Test organism:	Chinese Hamster cell line V79, clone 65/3
Origin:	Dr. 5.1.2.e Woo, Freiburg, Germany
Extrinsic metabolic activation system:	Post mitochondrial supernatant (S9 fraction) from Aroclor 1254 induced rat liver
Vehicle:	Bidistilled water
Growth medium:	The cells were maintained in Ham's F10 medium supplemented with 10% fetal calf serum and antibiotics.
Medium during treatment:	During the treatment period cells were maintained in growth medium in which the fetal calf serum was reduced to 3%. Antibiotics were omitted.
Selection medium:	The selection medium was growth medium to which 6-thioguanine (6-TG) was added to a final concentration of 8 μg/ml.

Concentrations:	***Cytotoxicity test*** Range with metabolic activation: 2.44 to 5000.0 µg/ml Range without metabolic activation: 2.44 to 5000.0 µg/ml ***Mutagenicity test*** **Original experiment**: Range with metabolic activation: 74.07 to 2000.0 µg/ml Range without metabolic activation: 37.04 to 1000.0 µg/ml **First confirmatory experiment**: Range with metabolic activation: 55.55 to 1500.0 µg/ml Range without metabolic activation: 37.04 to 1000.0 µg/ml **Second confirmatory experiment**: Range with metabolic activation: 400.0 to 1350.0 µg/ml Range without metabolic activation: 900.0 to 1200.0 µg/ml
Negative control:	Bidistilled water
Positive controls:	***With metabolic activation*** N-Nitrosodimethylamine (DMN), 1.0 µl/ml ***Without metabolic activation*** Ethylmethansulfonate (EMS), 0.3 µl/ml
Number of independent experiments:	3
Number of replicate cultures:	2
Testing facility:	Novartis Crop Protection AG CH-4002 Basel, Switzerland Genetic Toxicology
Location of archives	Novartis Crop Protection AG CH-4002 Basel, Switzerland Archives of Genetic Toxicology

Study Director:	Mr. 5.1.2.e Woo
Personnel:	***Technical conduct by*** Mrs. 5.1.2.e Woo Mr. 5.1.2.e Woo
Principal Investigator: (responsible for analytical study)	Dr. 5.1.2.e Woo Novartis Crop Protection AG CH-4002 Basel, Switzerland, Cell Biology
Sponsor:	Novartis Crop Protection AG CH-4002 Basel, Switzerland
Monitoring scientist:	Dr. 5.1.2.e Woo
Study initiation date:	June 29, 1998
Experimental start date:	August 18, 1998
Experimental termination date:	November 13, 1998
Study termination date:	December 03, 1998

ABSTRACT

CGA 108906 tech. (metabolite of CGA 329351), identified as white powder, 99%±2% purity, batch no. KI-5240/3 was tested for mutagenic effects on V79 Chinese hamster cells **in vitro**. The test substance was dissolved in bidistilled water. The cells were treated in the experiments with metabolic activation for 5 hours and in the experiments without metabolic activation for 21 hours. The results of each experiment were confirmed in a second and independent experiment (first confirmatory experiment). Since the test substance caused toxic effects in an extreme narrow concentration range, a second confirmatory experiment was performed with and without metabolic activation, to examine this range more in detail.

Cytotoxicity test
A preliminary range finding test was run assessing cytotoxicity. CGA 108906 tech. was tested at concentrations up to 5000.00 µg/ml. In the part with metabolic activation, the concentrations of 2500.00 and 5000.00 µg/ml were completely toxic, while the next lower concentration inhibited 78.6%. Without metabolic activation treatment with CGA 108906 tech. proved growth inhibiting down to the concentration of 1250.00 µg/ml. The next lower concentration revealed no acute inhibition of growth.
Accordingly, 2000.00 µg/ml with and 1000.00 µg/ml without metabolic activation were chosen as highest concentrations for the first mutagenicity assay.

Mutagenicity test with metabolic activation
The **original** experiment was performed at the following concentrations: 74.07, 222.22, 666.67 and 2000.00 µg/ml. After treatment the highest concentration was completely toxic. No toxicity was observed at the next lower concentration.
In the **first confirmatory** experiment the concentrations applied were 55.56, 166.67, 500.00 and 1500.00 µg/ml. The highest concentration was completely toxic. After treatment, the next lower concentration revealed a mean acute growth inhibition of 18.0%.
In the **second confirmatory** experiment the concentrations tested were 400.00, 600.00, 900.00 and 1350.00 µg/ml. The highest concentration revealed a mean acute growth inhibition of 99.3%. At subculture, this concentration was completely toxic. The next lower concentration revealed a mean acute growth inhibition of 22.8% after treatment,
N-Nitrosodimethylamine (DMN, 1.0 µl/ml) was used as positive control.
In all experiments comparison of the number of mutant colonies in the controls and in the cultures treated with the various concentrations of the test substance revealed no biological relevant increase of the mutant frequencies as determined by the screening with 6-TG.

Mutagenicity test without metabolic activation
The **original** experiment was performed at the following concentrations: 37.04, 111.11, 333.33 and 1000.00 µg/ml. The mean growth inhibition values found at the highest concentration after treatment and expression were 33.1% and 9.0% respectively.
In the **first confirmatory** experiment the concentrations applied were 37.04, 111.11, 333.33 and 1000.00 µg/ml. The highest concentration revealed a mean acute growth inhibitory effect of 52.2%. After expression, this concentration was not toxic.

In the **second confirmatory** experiment the concentrations tested were 900.00, 1000.00, 1100.00 and 1200.00 µg/ml. The mean growth inhibition values found after treatment were 91.2% at the highest concentration and 73.2% at the next lower one. After expression toxicity was markedly reduced at these concentrations.
Ethylmethansulfonate (EMS, 0.3 µl/ml) was used as positive control.
In all experiments comparison of the number of mutant colonies in the controls and in the cultures treated with the various concentrations of the test substance revealed no biological relevant increase of the mutant frequencies as determined by the screening with 6-TG.

CONCLUSION

Based on the results of two independently performed experiments and under the given experimental conditions, it is concluded that CGA 108906 tech. (metabolite of CGA 329351) and its metabolites did not show any mutagenic activity in this forward mutation system.

PROCEDURE

The test system allows the detection of base-pair substitutions, frameshift mutations and deletions induced by the test substance or by its metabolites [4,5]. Mutagenic effects are manifested by the appearance of cells resistant to 6-TG and can be quantified by comparison of the numbers of 6-TG resistant colonies in the treated and control cultures. To ensure that any mutagenic effect of metabolites of the test substance found in mammals is also detected, an experiment is performed, in which the metabolic turnover of the test material is simulated **in vitro** by the addition of an activation mixture to the cell cultures containing rat-liver post mitochondrial supernatant (S9 fraction) and co-factors [6-8].

Maintenance of the cell line
V79 Chinese hamster cells were originally derived from embryonic lung tissue. The cells were cultured in Ham's F10 medium supplemented with 10% pre-tested fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin in tissue culture (plastic) flasks. The humidity in the incubator was adjusted to >85% rH, the air was enriched to 5 ± 2.0 Vol% CO_2 and the temperature was 37±1°C. Twice per week the growth medium was replaced by fresh one.
The laboratory cultures were passaged weekly in low number (about $5x10^4$ cells per 175 cm^2) to keep the level of spontaneous mutants low and to prevent the cells of reaching a stationary phase of cell growth. Large stocks of the V79 cell line have been stored in liquid nitrogen allowing the repeated use of the same cell culture batch in experiments. Consequently, the parameters of the experiments remain similar because of the reproducible characteristics of the cells. The frozen cell suspension contains 10% dimethylsulfoxide (DMSO). All stock cells were cultured in cleansing medium for three days to purge the cultures of existing $hprt^-$ mutants. Cleansing medium was growth medium supplemented with 3 µM aminopterin. The cells have a stable karyotype with a modal chromosome number of 22±1. All stock cells were checked for mycoplasma contamination, using the Hoechst-Dye staining method or the 6-MPDR method, before being frozen. Thawed stock culture cells are kept not longer than for twelve passages (three months) in culture.

Solubilisation of the test substance
CGA 108906 tech. was dissolved in bidistilled water by aid of ultrasonication at room temperature and sterilised by filtration through a 0.2 µm filter. The highest concentration of CGA 108906 tech. was determined in a preliminary solubilisation test to be 400 mg/ml soluble in bidistilled water. Lower concentrations of the test substance were obtained by appropriate dilution of the stock solution with bidistilled water. The respective solutions were added 1:10 to the cell culture medium. The final concentration of bidistilled water in the culture medium was 10%. The test substance solutions were prepared immediately before the start of the test.

Preparation and composition of the metabolic activation mixture
Rat-liver post mitochondrial supernatant (S9 fraction) was prepared in advance from male rats (Tif:RAI/SPF), delivered by Animal Farm of BRL/CPB, Biological Research Laboratories Ltd., Füllinsdorf, 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 at 9000x g for 15 minutes and the resulting supernatant (S9 fraction) was stored

at approximately minus 80°C for no longer than one year. The protein content of the S9 fraction was mg/ml. S9 fraction was thawed immediately before use. The S9 mixture was prepared just prior use in an activation experiment and kept on ice [8]. The S9 mixture consisted of:

- Rat liver S9 fraction 250.0 µl/ml
- Glucose-6-phosphate 10.0 µmol/ml
- NADP 8.0 µmol/ml
- $CaCl_2$ 20.0 µmol/ml
- $MgCl_2$ 20.0 µmol/ml
- Na_2HPO_4 1.0 µmol/ml
- FCS 30.0 µl/ml

Unused portions of S9 fraction and S9 mixture were discarded and not saved for another experiment. The S9 mixture was immediately sterilised by filtration through a 0.2 µm filter. The activation mixture was added to the medium at a concentration of 10% in both the cytotoxicity test and the mutagenicity test and the final concentration of S9 fraction was 2.5% during the treatment.

Preliminary cytotoxicity test
A cytotoxicity test was performed on V79 cells as a preliminary test to determine the highest concentration of the test substance to be applied in the mutagenicity assay. For each concentration and the untreated controls, $2.5x10^5$ V79 cells were seeded in 5 ml growth medium into a 25 cm² tissue culture flask and incubated overnight. The cultures were exposed to the test substance for five hours in the presence and for 21 hours in the absence of a metabolic activation system. In the two parts of the experiment, 12 concentrations of the test substance and two vehicle (bidistilled water) controls were tested. The highest concentration was determined in a preliminary solubility test. Lower concentrations were prepared by serial dilution by a factor of 2. The treatment was terminated by washing the cultures with phosphate buffered saline (PBS). Compound-induced cytotoxicity was estimated by cloning efficiency immediately after treatment. The cultures were counted and diluted so that 100 cells were seeded per 9.6 cm² in 3 ml of growth medium. After seven to eight days of growth the cultures were fixed and stained with Giemsa and the surviving colonies determined with the aid of an electronic colony counter (Artek Counter®, Fisher Scientific) or by the naked eye. The sensitivity of the colony counter was adjusted to detect clones of about twenty or more cells. The concentration to be selected as the highest for the mutagenicity assay was the one causing about 50-90% reduction of viable cells in comparison with the mean of the two negative controls or corresponds to the substance's solubility limit (precipitates in the culture).

Mutagenicity test
Depending on the toxicity of the test compound $2.5\text{-}5.0x10^6$ cells of passage 24 (original experiment), passage 25 (first confirmatory experiment) and passage 28 (second confirmatory experiment) were plated in 30 ml growth medium into 175 cm² flasks and incubated overnight. The growth medium was replaced for five hours by 24 ml treatment medium and 3.0 ml S9 activation mixture, or for 21 hours by 27 ml treatment medium alone.

In each assay, cultures were treated in duplicate with four test chemical concentrations, a positive and a negative (bidistilled water) control. In the non-activated part of the experiment, the positive control was the ultimate mutagen Ethylmethansulphonate (EMS) at a concentration of 0.3 µl/ml. In the part with metabolic activation the positive control was the promutagen N-Nitrosodimethylamine (DMN) at a concentration of 1.0 µl/ml.

The treatment was terminated by washing the cell layer extensively with PBS. After washing, the cells were suspended by trypsinisation, pelleted, resuspended in fresh growth medium and counted with a haemocytometer or electronic coulter counter (Coulter Counter®, Model ZM), diluted with fresh growth medium and replated into flasks at $2x10^6$ cells. The cultures were incubated at 37°C for seven to eight days during which the cells could recover and divide to express the mutant phenotype. The cultures were subcultered after the second or third day transferring $2x10^6$ cells to a fresh flask to maintain exponential growth during the expression phase.

In parallel cytotoxicity of the compound was estimated from the cloning efficiency immediately after treatment. The counted cell suspension of each concentration level was further diluted so that 100 cells were seeded per 9.6 cm² in 2.5 ml of growth medium and incubated at 37°C. The number of colonies which developed within seven to eight days in these cultures reflected the viability at the end of the treatment (***survival values***).

At the end of the expression period the cultures were trypsinised, pelleted, resuspended in fresh growth medium and counted with a haemocytometer or electronic coulter counter (Coulter Counter®, Model ZM). The cell suspension of each culture was diluted with fresh growth medium and an aliquot replated into four flasks (75 cm² growth area) each containing $2x10^6$ cells for the mutant selection. The high-density cultures were subjected to the mutant selection procedure by supplementing the growth medium with 8 µg/ml 6-thioguanine (6-TG). Only cells mutated at the hprt locus could survive the 6-thioguanine treatment. The number of colonies formed in these flasks during the following days reflected the overall number of mutations induced by the treatment with the test substance or the mutagen (positive control). After seven to eight days incubation at 37°C, the cultures were fixed and stained with Giemsa. The mutant clones were counted with the naked eye.

In parallel the viability at the end of the expression period was estimated from the cloning efficiency. The remaining cell suspensions from the various expression cultures were further diluted such that 100 cells were seeded per 9.6 cm² in 2.5 ml of growth medium and were incubated at 37°C. The number of colonies which developed within these low-density cultures reflected the viability at the end of the expression period (***viability values***).

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 the analytical unit. This determination was performed with the lowest concentration of the stock solution used in the first and last segment of the mutagenicity test.

Assay acceptance criteria

- The results of the experiments should not be influenced by a technical error, contamination or a recognized artifact.
- From each experiment, at least three concentrations of the test substance, one positive and one solvent control should be evaluated.
- The mutant frequency of the solvent controls (spontaneous mutant frequency) should not exceed $35x10^{-6}$.
- The positive control should fulfill the criteria for a mutagenic substance.
- The highest concentration of the test substance applied in the mutagenicity test should either reduce the viable cells by about 50-90% or correspond to the test substance's solubility limit (precipitates in the culture). In case of non-toxic freely soluble compounds the highest tested concentration is 5 mg/ml. In special cases the highest concentration can be determined by the sponsor.

Assay evaluation criteria

All mutant frequencies are normalized to a virtual cloning efficiency of 100% at the end of the expression period. If the cloning efficiency of the viability cultures is lower than 15%, the corresponding mutant frequency is usually not calculated, since the results cannot be reasonably interpreted. For every concentration a mean mutant factor, which is defined as the ratio of the mean mutant frequencies of the treated cultures with the mean mutant frequencies of the solvent control cultures, is calculated.

Assessment of statistical significance of mutation frequency

Statistical significance of mutant frequencies (analysis of variance and test for linear trend) was carried out according to the UKEMS guidelines [9]

Criteria for a positive response

The test substance is considered to be mutagenic if:

- The assay is valid (see assay acceptance criteria)
- The mutant frequency at one or more concentrations is significantly greater than that of the negative control and the number of normalized mutant clones in the treated and untreated cultures differs by more than 20.
- There is a significant dose-relationship as indicated by the linear trend analysis.
- The effects described above are reproducible.

Exceptions

In extreme cases or if the results only partially satisfy the above criteria the Study Director (if necessary after consulting the person who will approve the report) will interpret the results from his own experience. Positive responses seen only at high levels of cytotoxicity will require careful interpretation when assessing their biological significance.

Historical negative and positive controls

The data of negative and positive controls from recent studies with this test system are shown in a table.

RESULTS

Toxicity

In the preliminary toxicity test with and without metabolic activation 12 concentrations of CGA 108906 tech. (metabolite of CGA 329351) were tested. The concentrations selected ranged from 2.44 to 5000.00 µg/ml and separated by 2-fold intervals. The values are shown in Table 1 and Table 2.
In the part with metabolic activation colony forming ability was completely inhibited at the two highest concentrations. The two lower concentrations exerted an acute toxcity of 78.6% and 43.4 %, respectively. In the part without metabolic activation CGA 108906 tech. exerted a practically complete growth inhibitory effect down to the concentration of 1250.00 µg/ml. The next lower concentration was not toxic.

Accordingly, four concentrations were selected for the original experiment ranging from 74.07 to 2000.00 µg/ml and from 37.04 to 1000.00 µg/ml in the presence and absence of metabolic activation, respectively.
In the part with metabolic activation, the concentration of 2000.00 µg/ml was completely toxic. No toxicity was observed at the next lower concentration.
In the absence of metabolic activation the mean growth inhibitory effect determined after treatment was 33.1% at the highest concentration. After the expression period the determined cytotoxicity revealed a mean value of 9.0%.
Due to the pronounced growth inhibitory response in the part with metabolic activation the concentration range was decreased in the first confirmatory experiment ranging from 55.55 to 1500.00 µg/ml. In the part without metabolic activation a concentration range of 37.04 to 1000.00 µg/ml was selected in order to reach a complete toxicity at that concentration. In the presence of metabolic activation the highest concentration was completely toxic. After treatment, the next lower concentration inhibited growth by 18.0%. In the part without activation, the mean growth inhibition at the highest concentration was 52.2%. After expression no toxic effect was visible at this concentration.
In order to perform the experiment at an optimal toxicity level, a second confirmatory experiment was performed testing the concentrations of 400.00 to 1350.00 with metabolic activation and 900.00, 1000.00, 1100.00, 1200.00 without metabolic activation. In the presence of metabolic activation the mean acute cytotoxicity at the highest concentration was 99.3%. At subculture, this concentration was completely toxic. The next lower concentration revealed a mean acute growth inhibition of 22.8% after treatment. In the part without activation, the highest concentration revealed a toxicity of 91.2%. The next lower concentration showed 73.2% toxicity. After expression, toxicity was markedly reduced at the these concentrations.

Mutagenicity

A summary of the results for the original, the first confirmatory and second confirmatory experiment is shown in Tables 3-8. The cytotoxicity determined after treatment, the number of viable cells at subculture, the cytotoxicity determined after the expression period and the mutant clones counted are shown in Tables 9-12 (original experiment, with metabolic activation), Tables 13-16 (original experiment, without metabolic activation), Tables 17-20 (first confirmatory experiment,

with metabolic activation), Tables 21-24 (first confirmatory experiment, without metabolic activation), Tables 25-28 (second confirmatory experiment, with metabolic activation) and Tables 29-32 (second confirmatory experiment, without metabolic activation).

In the second confirmatory experiment with metabolic activation, a marginal, but statistically significant increased mutant frequency was observed at the concentration of 400.0 µg/ml (Table 7). This effect, however, showed no dose-relationship and was not seen in the other experiments with activation. Furthermore it did not fulfill the criteria for a positive response (the number of normalized mutant clones in the treated and untreated cultures does not differ by more than 20). The effect is therefore considered to be of spontaneous origin and not related to treatment with the test material. None of the other values showed a statistically significant difference when compared with their respective negative control.

In the presence and absence of metabolic activation, no biological relevant increase in mutant frequency was observed at any concentration level of CGA 108906 tech. (metabolite of CGA 329351) in comparison with the negative control.

The positive controls induced a clear increase in mutant frequency.

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 stock solution and the lowest concentration of the test substance in solution were performed by HPLC with UV detection at 229 nm. 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 vecile.

DEVIATIONS TO PROTOCOL

The OECD guideline has been adopted on July 21, 1997.

The EPA guideline has been adopted in August, 1998.

REFERENCES

1) OECD (July 21, 1997)
Genetic Toxicology: In vitro Mammalian Cell Gene Mutation Tests.
OECD Guideline for Testing of Chemicals 476.

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Health Effects Test Guidelines
OPPTS 870.5300
In vitro Mammalian Cell Gene Mutation Test

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HISTORICAL CONTROL DATA FROM GENE MUTATION TESTS WITH CHINESE HAMSTER CELLS V79

Historical control data V79/hprt gene mutation test

Test no.	Mutant frequency values (x10E-6)			
	With activation		Without activation	
	Negative control	Positive control (DMN 1.0 µl/ml)	Negative control	Positive control (EMS 0.3 µl/ml)
920837/0	5.69	295.86	3.17	1566.48
920837/1	8.74	157.67	2.92	1787.97
926186/0	7.52	214.40	5.82	1953.78
926186/1	10.61	272.94	4.86	390.83
926283/0	14.10	105.21	21.48	1047.93
926283/1	2.11	194.35	2.28	1799.31
926249/0	4.08	125.47	1.26	432.96
926249/1	4.38	101.64	4.35	1350.97
926298/0	1.21	149.65	0.76	1692.18
926298/1	3.80	141.77	2.54	1527.79
921198/0	4.63	84.50	6.51	1785.63
921198/1	2.30	126.00	1.91	1925.66
923168/0	2.07	94.84	3.34	1684.16
923168/1	5.05	77.39	3.85	1798.01
932805/0	8.11	200.67	3.99	1941.84
932805/1	1.83	127.52	1.88	1942.32
936016/0	3.25	148.12	4.36	2160.86
936016/1	2.80	150.39	2.92	1927.38
936096/0	15.42	164.53	10.74	1505.88
936096/1	8.57	119.35	6.49	2151.83
923141/0	9.13	118.90	5.43	1694.51
923141/1	3.48	148.11	1.51	2062.83
936100/0	6.57	130.73	2.05	1751.05
936100/1	1.79	121.95	1.97	1988.61
936091/0	6.44	148.77	10.93	1963.33
936091/1	5.47	97.88	2.97	1549.36
931116/0	1.57	117.04	1.69	1405.97
931116/1	2.99	112.80	3.15	1425.14
934040/0	1.01	127.63	4.33	2274.64
934040/1	9.97	153.03	8.11	2010.85
934083/0	3.18	174.74	3.78	1868.34
934083/1	2.13	160.71	2.89	1454.39
936095/0	2.41	125.24	2.82	1552.94
936095/1	5.96	145.84	3.11	1823.21
936136/0	13.29	150.63	19.38	2120.24
936136/1	3.08	128.34	1.08	1761.19
936153/0	3.26	135.31	1.64	2109.38
936153/1	1.91	146.10	4.05	1947.51

Historical control data V79/hprt gene mutation test				
	Mutant frequency values (x10E-6)			
	With activation		Without activation	
Test no.	Negative control	Positive control (DMN 1.0 µl/ml)	Negative control	Positive control (EMS 0.3 µl/ml)
936220/0	5.96	123.58	4.97	1500.00
936220/1	9.26	147.26	11.43	2185.09
944017/0	9.47	155.23	11.81	2050.74
944017/1	11.59	142.03	5.61	1920.50
946018/0	2.51	137.61	2.05	1907.66
946018/1	3.34	138.79	1.32	2111.63
946006/0	6.51	116.68	3.51	1545.58
946006/1	1.86	95.16	1.93	1779.79
946001/0	4.03	118.53	1.75	1791.20
946001/1	9.69	125.97	14.03	1972.41
946036/0	15.68	174.71	15.30	3099.81
946036/1	14.37	158.56	18.48	2841.34
940846/0	15.13	148.74	11.17	2409.35
940846/1	3.56	138.59	4.07	1845.49
940848/0	3.03	149.47	9.51	2055.58
940848/1	6.07	137.36	2.96	2481.73
946192/0	3.19	126.99	5.11	2557.69
941079/0	2.71	145.43	3.41	2604.33
941079/1	7.12	166.40	4.04	2831.63
940845/0	2.35	169.59	3.08	1764.06
940845/1	6.38	142.43	4.82	2292.44
940852/0	3.33	162.06	4.97	1515.17
940852/1	3.83	132.98	5.03	1690.63
940847/0	6.46	165.06	2.65	1890.35
940847/1	7.50	203.61	8.02	2638.32
940850/0	3.71	222.12	3.81	2030.44
940850/1	4.02	168.10	4.20	1720.75
940849/0	9.94	161.78	13.29	2162.02
940849/1	9.22	147.20	8.93	1688.82
940855/0	5.91	134.67	6.40	2117.21
940855/1	5.28	152.03	5.14	2283.07
940851/0	7.82	189.37	5.13	1623.84
940851/1	4.96	209.64	2.66	2545.29
940854/0	4.16	127.85	3.06	2461.51
940854/1	1.42	179.83	3.45	2031.18
941008/0	2.82	140.73	2.36	1806.8
941008/1	4.3	140.83	3.58	1648.64
943075/0	14.38	206.33	12.86	2897.43
943075/1	14.17	152.71	8.96	2686.18
944081/0	4.44	147.11	4.3	2190.17
944081/1	6.59	142.59	4.54	1906.1

Test no.	Mutant frequency values (x10E-6)			
	With activation		Without activation	
	Negative control	Positive control (DMN 1.0 µl/ml)	Negative control	Positive control (EMS 0.3 µl/ml)
944083/0	5.44	170.82	5.25	2587.76
944083/1	3.54	190.92	6.65	2069.69
944101/0	8.65	202.72	2.68	2001.45
944101/1	4.72	118.83	3.03	1622.43
946029/0	4.01	166.31	4.82	2238.46
946029/1	4.60	197.42	2.89	2535.92
946171/0	4.41	147.48	5.48	1561.51
946171/1	4.37	131.67	3.99	1903.93
946207/0	5.91	164.15	8.35	2529.52
946207/1	6.22	124.15	5.81	1494.05
950807/0	7.57	115.47	4.88	1450.65
950807/1	2.50	121.52	1.69	1310.62
950808/0	6.75	145.42	5.65	1684.98
950808/1	6.11	113.89	3.08	1650.69
950810/0	3.34	120.05	4.84	1664.19
950810/1	2.92	100.46	2.66	1330.94
950811/0	2.37	119.42	4.11	1538.05
950811/1	4.73	128.32	4.26	1600.96
954000/0	13.17	172.14	13.78	2203.81
954000/1	7.26	115.86	7.48	1147.31
954002/0	8.43	126.87	6.94	2071.90
954002/1	4.70	99.40	5.33	1481.06
956038/0	3.94	132.22	3.56	1977.86
956038/1	3.94	123.06	6.18	1528.85
956041/0	6.71	115.93	5.94	1708.82
956041/1	5.39	131.62	4.42	1594.94
956122/0	5.07	110.78	2.09	1744.05
956122/1	4.59	98.44	3.92	1901.66
950820/0	4.15	134.75	3.05	1377.21
950820/1	5.24	91.58	7.03	1580.27
950821/0	7.11	90.27	3.70	1221.85
950821/1	4.40	117.24	3.30	1620.82
950823/0	4.69	117.18	5.05	1413.76
950823/1	6.69	126.91	6.32	1330.18
952015/0	6.98	120.76	8.01	1514.49
952015/1	3.36	122.87	4.00	1748.34
956105/0	5.02	72.87	4.12	1768.65
956105/1	5.02	134.72	3.47	1962.66
956097/0	7.70	117.79	4.61	2218.36
956097/1	5.22	88.33	7.63	1650.00
956178/0	5.49	85.38	5.24	2440.44
956178/1	9.21	92.77	6.26	1310.34

Test no.	Mutant frequency values (x10E-6)			
	With activation		Without activation	
	Negative control	Positive control (DMN 1.0 µl/ml)	Negative control	Positive control (EMS 0.3 µl/ml)
951136/0	2.75	96.51	3.31	1604.66
951136/1	6.95	108.80	9.65	1338.01
956101/0	10.32	124.85	9.79	1635.50
956101/1	5.11	107.61	6.38	1703.52
951077/0	6.52	110.43	3.99	1024.78
951077/1	6.16	88.66	6.10	1238.56
956145/0	3.21	96.72	3.40	1256.03
956145/1	7.63	110.20	6.90	1072.92
956208/0	5.13	104.48	4.27	1482.19
956208/1	3.71	102.95	4.22	1432.75
963056/0	2.68	101.21	3.51	1345.04
963056/1	1.16	94.95	2.66	1182.32
966045/0	3.23	105.14	3.97	1807.23
966045/1	3.39	95.51	3.29	1402.50
966103/0	2.34	87.42	3.45	1330.97
966103/1	2.78	94.78	3.67	1387.50
964403/0	3.01	93.90	2.71	1243.41
964403/1	2.91	91.73	3.15	1480.85
962807/0	2.73	96.55	3.19	1466.24
962807/1	2.49	97.55	3.04	1432.91
966156/0	2.62	88.42	3.25	1604.00
966156/1	3.16	108.64	3.63	1550.78
966150/0	2.19	121.60	2.94	1298.90
966150/1	2.08	100.37	2.54	891.00
971024/0	3.34	208.22	1.60	768.58
971024/1	2.15	114.76	2.59	600.53
971059/0	4.33	66.50	2.42	757.41
971059/1	6.01	145.06	8.62	686.79
966146/0	2.85	92.69	5.12	1418.70
966146/1	3.19	98.57	5.52	1585.29
971092/0	7.49	153.44	1.19	963.96
971092/1	12.42	84.83	6.80	700.43
	153	*153*	*153*	*153*
Mean	*5.46*	*133.68*	*5.09*	*1739.91*
±S.D.	*3.24*	*36.40*	*3.48*	*479.10*
Min.	*1.01*	*66.50*	*0.76*	*390.83*
Max.	*15.68*	*295.86*	*21.48*	*3099.81*

LEGEND TO TABLES 1 TO 32

*	No data
Tx	No data due to high toxicity
nTx	Not toxic
§	One duplicate lost
Ns	Not significant

(All calculations on the following tables were made by a computer using exact values. The calculated values given in the tables are rounded to two or three digits.)

TABLE 1 : RESULT OF THE CYTOTOXICITY TEST
Experiment with metabolic activation

Test number : 983071
Experiment : Original
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number after treatment (x10E6)	Survival clones after treatment (per well)					
Negative control	0.512	67	78	64	66	69	67
Negative control	0.560	64	73	69	76	61	80
CGA 108 906 tech.:							
5000.0000 µg/ml	0.035	Tx	Tx	Tx	Tx	Tx	Tx
2500.0000 µg/ml	0.021	0	0	0	0	0	0
1250.0000 µg/ml	0.299	26	27	21	27	33	26
625.0000 µg/ml	0.558	45	31	26	43	37	45
312.5000 µg/ml	0.549	82	72	65	78	70	71
156.2500 µg/ml	0.528	73	63	59	73	65	73
78.1250 µg/ml	0.508	66	62	71	65	77	81
39.0625 µg/ml	0.417	78	62	77	60	57	62
19.5313 µg/ml	0.609	50	46	50	68	54	65
9.7656 µg/ml	0.532	68	84	61	66	50	51
4.8828 µg/ml	0.539	64	65	70	71	75	76
2.4414 µg/ml	0.595	64	71	67	58	61	57

Treatment	Mean of clones	Number of viable cells (x10E6)	Acute cytotoxicity (% of control)
Negative control	68.50	0.35	
Negative control	70.50	0.39	
CGA 108 906 tech.:			
5000.0000 µg/ml	*	*	*
2500.0000 µg/ml	*	*	*
1250.0000 µg/ml	26.67	0.08	78.61
625.0000 µg/ml	37.83	0.21	43.35
312.5000 µg/ml	73.00	0.40	nTx
156.2500 µg/ml	67.67	0.36	4.14
78.1250 µg/ml	70.33	0.36	4.11
39.0625 µg/ml	66.00	0.28	26.10
19.5313 µg/ml	55.50	0.34	9.29
9.7656 µg/ml	63.33	0.34	9.50
4.8828 µg/ml	70.17	0.38	nTx
2.4414 µg/ml	63.00	0.37	nTx

TABLE 2 : RESULT OF THE CYTOTOXICITY TEST
Experiment without metabolic activation

Test number : 983071
Experiment : Original
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number after treatment (x10E6)	Survival clones after treatment (per well)					
Negative control	0.718	94	96	98	92	94	95
Negative control	0.730	98	96	98	95	94	92
CGA 108 906 tech.:							
5000.0000 µg/ml	0.014	Tx	Tx	Tx	Tx	Tx	Tx
2500.0000 µg/ml	0.060	1	0	0	2	2	1
1250.0000 µg/ml	0.441	5	7	8	2	8	6
625.0000 µg/ml	0.976	89	85	85	91	95	88
312.5000 µg/ml	0.917	82	89	94	82	95	91
156.2500 µg/ml	0.940	82	88	76	72	82	85
78.1250 µg/ml	0.934	87	84	72	90	86	88
39.0625 µg/ml	0.711	83	85	88	89	80	82
19.5313 µg/ml	0.903	79	84	82	84	78	89
9.7656 µg/ml	0.900	88	84	82	91	83	82
4.8828 µg/ml	0.814	89	88	92	91	85	94
2.4414 µg/ml	0.733	93	94	89	84	93	91

Treatment	Mean of clones	Number of viable cells (x10E6)	Acute cytotoxicity (% of control)
Negative control	94.83	0.68	
Negative control	95.50	0.70	
CGA 108 906 tech.:			
5000.0000 µg/ml	*	*	*
2500.0000 µg/ml	1.00	0.00	99.91
1250.0000 µg/ml	6.00	0.03	96.16
625.0000 µg/ml	88.83	0.87	nTx
312.5000 µg/ml	88.83	0.81	nTx
156.2500 µg/ml	80.83	0.76	nTx
78.1250 µg/ml	84.50	0.79	nTx
39.0625 µg/ml	84.50	0.60	12.81
19.5313 µg/ml	82.67	0.75	nTx
9.7656 µg/ml	85.00	0.76	nTx
4.8828 µg/ml	89.83	0.73	nTx
2.4414 µg/ml	90.67	0.66	3.56

TABLE 3 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment with metabolic activation

Test number : 983071
Experiment : Original
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	64.25	4.25	6.61
Positive control DMN 1 μl/ml	51.00	98.25	192.65
CGA 108 906 tech.:			
2000.0000 μg/ml	*	*	*
666.6667 μg/ml	67.83	3.50	5.16
222.2222 μg/ml	69.92	3.75	5.36
74.0741 μg/ml	78.92	2.88	3.64

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	3.31		
Positive control DMN 1 μl/ml	96.32	29.12	P<0.001
CGA 108 906 tech.:			
2000.0000 μg/ml	*	*	*
666.6667 μg/ml	2.58	0.78	Ns
222.2222 μg/ml	2.68	0.81	Ns
74.0741 μg/ml	1.82	0.55	Ns

Linear relation: Ns

TABLE 4 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment without metabolic activation

Test number : 983071
Experiment : Original
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	45.00	3.50	7.78
Positive control EMS 0.3 µl/ml	36.17	931.00	2574.19
CGA 108 906 tech.:			
1000.0000 µg/ml	42.25	4.00	9.47
333.3333 µg/ml	48.17	2.75	5.71
111.1111 µg/ml	44.50	3.50	7.87
37.0370 µg/ml	40.58	2.75	6.78

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	3.89		
Positive control EMS 0.3 µl/ml	1287.10	330.97	P<0.001
CGA 108 906 tech.:			
1000.0000 µg/ml	4.73	1.22	Ns
333.3333 µg/ml	2.85	0.73	Ns
111.1111 µg/ml	3.93	1.01	Ns
37.0370 µg/ml	3.39	0.87	Ns

Linear relation: Ns

TABLE 5 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment with metabolic activation

Test number : 983071
Experiment : 1st Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	71.25	6.13	8.60
Positive control DMN 1 µl/ml	48.17	119.38	247.84
CGA 108 906 tech.:			
1500.0000 µg/ml	*	*	*
500.0000 µg/ml	69.42	4.50	6.48
166.6667 µg/ml	68.92	3.75	5.44
55.5556 µg/ml	75.17	5.13	6.82

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	4.30		
Positive control DMN 1 µl/ml	123.92	28.83	P<0.001
CGA 108 906 tech.:			
1500.0000 µg/ml	*	*	*
500.0000 µg/ml	3.24	0.75	Ns
166.6667 µg/ml	2.72	0.63	Ns
55.5556 µg/ml	3.41	0.79	Ns

Linear relation: Ns

TABLE 6 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment without metabolic activation

Test number : 983071
Experiment : 1st Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	42.17	2.25	5.34
Positive control EMS 0.3 µl/ml	49.00	971.75	1983.16
CGA 108 906 tech.:			
1000.0000 µg/ml	49.92	2.13	4.26
333.3333 µg/ml	53.42	2.13	3.98
111.1111 µg/ml	42.00	3.13	7.44
37.0370 µg/ml	52.25	1.63	3.11

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	2.67		
Positive control EMS 0.3 µl/ml	991.58	371.66	P<0.001
CGA 108 906 tech.:			
1000.0000 µg/ml	2.13	0.80	Ns
333.3333 µg/ml	1.99	0.75	Ns
111.1111 µg/ml	3.72	1.39	Ns
37.0370 µg/ml	1.56	0.58	Ns

Linear relation: Ns

TABLE 7 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment with metabolic activation

Test number : 983071
Experiment : 2nd Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	74.82	3.38	4.51
Positive control DMN 1 µl/ml	69.42	126.00	181.51
CGA 108 906 tech.:			
1350.0000 µg/ml	*	*	*
900.0000 µg/ml	77.42	2.38	3.07
600.0000 µg/ml	74.92	3.00	4.00
400.0000 µg/ml	78.33	6.75	8.62

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	2.26		
Positive control DMN 1 µl/ml	90.76	40.24	P<0.001
CGA 108 906 tech.:			
1350.0000 µg/ml	*	*	*
900.0000 µg/ml	1.53	0.68	Ns
600.0000 µg/ml	2.00	0.89	Ns
400.0000 µg/ml	4.31	1.91	0.02<P<0.05

Linear relation: Ns

TABLE 8 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment without metabolic activation

Test number : 983071
Experiment : 2nd Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	49.33	4.00	8.11
Positive control EMS 0.3 µl/ml	47.33	897.88	1896.92
CGA 108 906 tech.:			
1200.0000 µg/ml	60.42	4.63	7.66
1100.0000 µg/ml	31.83	4.25	13.35
1000.0000 µg/ml	32.08	2.50	7.79
900.0000 µg/ml	57.42	3.38	5.88

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	4.05		
Positive control EMS 0.3 µl/ml	948.46	233.95	P<0.001
CGA 108 906 tech.:			
1200.0000 µg/ml	3.83	0.94	Ns
1100.0000 µg/ml	6.68	1.65	Ns
1000.0000 µg/ml	3.90	0.96	Ns
900.0000 µg/ml	2.94	0.72	Ns

Linear relation: Ns

TABLE 9 : CYTOTOXICITY DETERMINED AFTER TREATMENT
Experiment with metabolic activation

Test number : 983071
Experiment : Original
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	**Cell number after treat-ment (x10E6)**	**Survival clones after treatment (per well)**					
Negative control	7.63	56	55	59	56	75	54
Negative control	6.17	66	54	72	54	55	62
Positive control:							
DMN 1 µl/ml	7.36	69	71	65	72	65	65
DMN 1 µl/ml	7.52	89	86	79	79	89	79
CGA 108 906 tech.:							
2000.0000 µg/ml	0.23	Tx	Tx	Tx	Tx	Tx	Tx
2000.0000 µg/ml	0.26	Tx	Tx	Tx	Tx	Tx	Tx
666.6667 µg/ml	6.16	70	61	84	71	66	70
666.6667 µg/ml	7.38	73	64	65	53	72	66
222.2222 µg/ml	8.47	66	55	75	66	75	72
222.2222 µg/ml	7.67	66	66	60	59	61	71
74.0741 µg/ml	7.95	54	64	54	39	40	49
74.0741 µg/ml	8.17	60	47	75	59	61	47

Treatment	**Mean of survival clones**	**Number of viable cells (x10E6)**	**Acute cyto-toxicity (% of control)**
Negative control	59.17	4.51	
Negative control	60.50	3.73	
Positive control:			
DMN 1 µl/ml	67.83	4.99	nTx
DMN 1 µl/ml	83.50	6.28	nTx
CGA 108 906 tech.:			
2000.0000 µg/ml	*	*	*
2000.0000 µg/ml	*	*	*
666.6667 µg/ml	70.33	4.33	nTx
666.6667 µg/ml	65.50	4.83	nTx
222.2222 µg/ml	68.17	5.77	nTx
222.2222 µg/ml	63.83	4.90	nTx
74.0741 µg/ml	50.00	3.98	3.59
74.0741 µg/ml	58.17	4.75	nTx

TABLE 10 : NUMBER OF VIABLE CELLS AT SUBCULTURE
Experiment with metabolic activation

Test number : 983071
Experiment : Original
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	**Cell number at subculture (x10E6)**	**% growth of control**
Negative control	15.48	
Negative control	14.40	
Positive control:		
DMN 1 µl/ml	13.33	89.20
DMN 1 µl/ml	15.42	103.21
<u>CGA 108 906 tech.:</u>		
2000.0000 µg/ml	Tx	*
2000.0000 µg/ml	Tx	*
666.6667 µg/ml	14.93	99.95
666.6667 µg/ml	15.90	106.44
222.2222 µg/ml	16.89	113.07
222.2222 µg/ml	18.25	122.16
74.0741 µg/ml	16.92	113.27
74.0741 µg/ml	15.30	102.38

TABLE 11 : CYTOTOXICITY DETERMINED AFTER EXPRESSION
Experiment with metabolic activation

Test number : 983071
Experiment : Original
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number after expression (x10E6)	Viability clones after expression (per well)					
Negative control	17.34	53	53	64	57	67	61
Negative control	18.02	62	72	54	74	75	79
Positive control:							
DMN 1 µl/ml	16.52	41	59	46	66	64	58
DMN 1 µl/ml	13.42	45	43	42	58	50	40
CGA 108 906 tech.:							
2000.0000 µg/ml	Tx	Tx	Tx	Tx	Tx	Tx	Tx
2000.0000 µg/ml	Tx	Tx	Tx	Tx	Tx	Tx	Tx
666.6667 µg/ml	17.98	61	74	68	64	67	63
666.6667 µg/ml	17.24	64	72	74	66	65	76
222.2222 µg/ml	18.14	75	51	72	73	76	72
222.2222 µg/ml	16.59	73	78	81	83	50	55
74.0741 µg/ml	16.58	86	79	79	88	86	67
74.0741 µg/ml	15.53	72	83	89	82	72	64

Treatment	Mean of viability clones	Number of viable cells (x10E6)	Cytotoxicity (% of control)
Negative control	59.17	10.26	
Negative control	69.33	12.49	
Positive control:			
DMN 1 µl/ml	55.67	9.19	19.18
DMN 1 µl/ml	46.33	6.22	45.35
CGA 108 906 tech.:			
2000.0000 µg/ml	*	*	*
2000.0000 µg/ml	*	*	*
666.6667 µg/ml	66.17	11.89	nTx
666.6667 µg/ml	69.50	11.98	nTx
222.2222 µg/ml	69.83	12.67	nTx
222.2222 µg/ml	70.00	11.61	nTx
74.0741 µg/ml	80.83	13.40	nTx
74.0741 µg/ml	77.00	11.96	nTx

TABLE 12 : 6-TG RESISTANT (MUTANT) CLONES COUNTED
Experiment with metabolic activation

Test number : 983071
Experiment : Original
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	6-Thioguanine resistant mutant clones counted (per flask)				Mean of the mutant clones counted
Negative control	1	3	6	4	3.50
Negative control	4	5	6	5	5.00
Positive control:					
DMN 1 µl/ml	102	106	112	102	105.50
DMN 1 µl/ml	94	80	96	94	91.00
CGA 108 906 tech.:					
2000.0000 µg/ml	Tx	Tx	Tx	Tx	*
2000.0000 µg/ml	Tx	Tx	Tx	Tx	*
666.6667 µg/ml	1	4	5	5	3.75
666.6667 µg/ml	2	2	4	5	3.25
222.2222 µg/ml	6	7	3	6	5.50
222.2222 µg/ml	2	1	2	3	2.00
74.0741 µg/ml	5	3	1	2	2.75
74.0741 µg/ml	2	2	5	3	3.00

TABLE 13 : CYTOTOXICITY DETERMINED AFTER TREATMENT
Experiment without metabolic activation

Test number : 983071
Experiment : Original
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	**Cell number after treat-ment (x10E6)**	**Survival clones after treatment (per well)**					
Negative control	17.47	71	91	94	82	95	95
Negative control	17.18	92	98	95	98	98	96
Positive control:							
EMS 0.3 µl/ml	15.29	80	79	92	86	83	84
EMS 0.3 µl/ml	15.78	80	93	94	101	84	85
<u>**CGA 108 906 tech.:**</u>							
1000.0000 µg/ml	12.14	105	93	96	83	88	77
1000.0000 µg/ml	11.67	89	87	85	105	80	88
333.3333 µg/ml	15.74	85	98	93	96	91	96
333.3333 µg/ml	17.33	96	92	102	93	104	96
111.1111 µg/ml	17.60	95	98	93	98	95	98
111.1111 µg/ml	18.64	87	98	98	93	97	95
37.0370 µg/ml	17.44	99	98	97	96	97	98
37.0370 µg/ml	18.17	94	97	98	99	99	98

Treatment	**Mean of survival clones**	**Number of viable cells (x10E6)**	**Acute cyto-toxicity (% of control)**
Negative control	88.00	15.37	
Negative control	96.17	16.52	
Positive control:			
EMS 0.3 µl/ml	84.00	12.84	19.49
EMS 0.3 µl/ml	89.50	14.12	11.45
<u>**CGA 108 906 tech.:**</u>			
1000.0000 µg/ml	90.33	10.96	31.27
1000.0000 µg/ml	89.00	10.38	34.91
333.3333 µg/ml	93.17	14.66	8.07
333.3333 µg/ml	97.17	16.84	nTx
111.1111 µg/ml	96.17	16.92	nTx
111.1111 µg/ml	94.67	17.64	nTx
37.0370 µg/ml	97.50	17.00	nTx
37.0370 µg/ml	97.50	17.72	nTx

TABLE 14 : NUMBER OF VIABLE CELLS AT SUBCULTURE
Experiment without metabolic activation

Test number : 983071
Experiment : Original
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number at subculture (x10E6)	% growth of control
Negative control	15.92	
Negative control	13.79	
Positive control:		
EMS 0.3 µl/ml	10.49	70.62
EMS 0.3 µl/ml	11.08	74.60
CGA 108 906 tech.:		
1000.0000 µg/ml	11.94	80.37
1000.0000 µg/ml	11.55	77.72
333.3333 µg/ml	14.85	99.97
333.3333 µg/ml	15.02	101.07
111.1111 µg/ml	14.75	99.28
111.1111 µg/ml	15.26	102.70
37.0370 µg/ml	13.58	91.42
37.0370 µg/ml	14.37	96.73

TABLE 15 : CYTOTOXICITY DETERMINED AFTER EXPRESSION
Experiment without metabolic activation

Test number : 983071
Experiment : Original
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number after expression (x10E6)	Viability clones after expression (per well)					
Negative control	16.40	46	42	42	40	34	37
Negative control	17.18	40	44	63	55	40	57
Positive control:							
EMS 0.3 µl/ml	12.71	26	25	42	28	35	24
EMS 0.3 µl/ml	12.72	36	47	37	52	42	40
CGA 108 906 tech.:							
1000.0000 µg/ml	17.31	43	36	37	33	39	41
1000.0000 µg/ml	15.50	50	45	35	42	54	52
333.3333 µg/ml	16.00	51	59	56	38	40	43
333.3333 µg/ml	18.19	50	52	51	56	47	35
111.1111 µg/ml	18.54	45	40	56	51	51	48
111.1111 µg/ml	18.98	48	40	47	38	31	39
37.0370 µg/ml	17.00	31	39	39	31	47	29
37.0370 µg/ml	16.09	45	57	44	34	52	39

Treatment	Mean of viability clones	Number of viable cells (x10E6)	Cytotoxicity (% of control)
Negative control	40.17	6.59	
Negative control	49.83	8.56	
Positive control:			
EMS 0.3 µl/ml	30.00	3.81	49.64
EMS 0.3 µl/ml	42.33	5.38	28.93
CGA 108 906 tech.:			
1000.0000 µg/ml	38.17	6.61	12.79
1000.0000 µg/ml	46.33	7.18	5.18
333.3333 µg/ml	47.83	7.66	nTx
333.3333 µg/ml	48.50	8.82	nTx
111.1111 µg/ml	48.50	8.99	nTx
111.1111 µg/ml	40.50	7.69	nTx
37.0370 µg/ml	36.00	6.12	19.20
37.0370 µg/ml	45.17	7.27	4.04

TABLE 16 : 6-TG RESISTANT (MUTANT) CLONES COUNTED
Experiment without metabolic activation

Test number : 983071
Experiment : Original
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	6-Thioguanine resistant mutant clones counted (per flask)				Mean of the mutant clones counted
Negative control	6	3	6	4	4.75
Negative control	2	3	1	3	2.25
Positive control:					
EMS 0.3 µl/ml	897	854	890	906	886.75
EMS 0.3 µl/ml	992	964	961	984	975.25
CGA 108 906 tech.:					
1000.0000 µg/ml	2	2	2	2	2.00
1000.0000 µg/ml	6	7	4	7	6.00
333.3333 µg/ml	6	2	1	1	2.50
333.3333 µg/ml	4	4	3	1	3.00
111.1111 µg/ml	7	1	6	3	4.25
111.1111 µg/ml	3	3	2	3	2.75
37.0370 µg/ml	1	1	2	5	2.25
37.0370 µg/ml	3	3	5	2	3.25

TABLE 17 : CYTOTOXICITY DETERMINED AFTER TREATMENT
Experiment with metabolic activation

Test number : 983071
Experiment : 1st Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number after treat-ment (x10E6)	Survival clones after treatment (per well)					
Negative control	12.38	56	52	66	67	71	75
Negative control	12.10	69	66	61	62	67	58
Positive control:							
DMN 1 µl/ml	11.79	94	93	81	97	97	96
DMN 1 µl/ml	12.46	70	60	61	55	60	52
CGA 108 906 tech.:							
1500.0000 µg/ml	1.28	Tx	Tx	Tx	Tx	Tx	Tx
1500.0000 µg/ml	0.88	Tx	Tx	Tx	Tx	Tx	Tx
500.0000 µg/ml	12.02	50	54	57	50	49	51
500.0000 µg/ml	11.53	65	60	66	56	47	52
166.6667 µg/ml	11.83	72	55	61	59	47	56
166.6667 µg/ml	12.22	48	43	46	59	63	54
55.5556 µg/ml	11.38	41	46	38	47	48	34
55.5556 µg/ml	12.25	55	75	68	58	73	51

Treatment	Mean of survival clones	Number of viable cells (x10E6)	Acute cyto-toxicity (% of control)
Negative control	64.50	7.99	
Negative control	63.83	7.73	
Positive control:			
DMN 1 µl/ml	93.00	10.96	nTx
DMN 1 µl/ml	59.67	7.43	5.40
CGA 108 906 tech.:			
1500.0000 µg/ml	*	*	*
1500.0000 µg/ml	*	*	*
500.0000 µg/ml	51.83	6.23	20.69
500.0000 µg/ml	57.67	6.65	15.37
166.6667 µg/ml	58.33	6.90	12.14
166.6667 µg/ml	52.17	6.37	18.88
55.5556 µg/ml	42.33	4.82	38.67
55.5556 µg/ml	63.33	7.76	1.27

TABLE 18 : NUMBER OF VIABLE CELLS AT SUBCULTURE
Experiment with metabolic activation

Test number : 983071
Experiment : 1st Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	**Cell number at subculture (x10E6)**	**% growth of control**
Negative control	15.36	
Negative control	12.44	
Positive control:		
DMN 1 µl/ml	9.45	67.97
DMN 1 µl/ml	10.60	76.24
<u>**CGA 108 906 tech.:**</u>		
1500.0000 µg/ml	0.19	1.35
1500.0000 µg/ml	0.33	2.38
500.0000 µg/ml	14.02	100.89
500.0000 µg/ml	14.84	106.78
166.6667 µg/ml	15.48	111.40
166.6667 µg/ml	14.21	102.25
55.5556 µg/ml	14.69	105.71
55.5556 µg/ml	14.34	103.18

TABLE 19 : CYTOTOXICITY DETERMINED AFTER EXPRESSION
Experiment with metabolic activation

Test number	:	**983071**
Experiment	:	**1st Confirmatory**
Test substance	:	**CGA 108 906 tech.**
Batch	:	**KI-5240/3**

Treatment	**Cell number after expres-sion (x10E6)**	**Viability clones after expression (per well)**					
Negative control	19.10	62	72	92	84	79	78
Negative control	20.10	63	67	67	62	72	57
Positive control:							
DMN 1 µl/ml	13.47	48	45	48	47	47	40
DMN 1 µl/ml	14.84	50	38	52	64	55	44
CGA 108 906 tech.:							
1500.0000 µg/ml	Tx	Tx	Tx	Tx	Tx	Tx	Tx
1500.0000 µg/ml	Tx	Tx	Tx	Tx	Tx	Tx	Tx
500.0000 µg/ml	19.17	89	71	78	62	70	66
500.0000 µg/ml	19.72	66	65	76	59	58	73
166.6667 µg/ml	20.69	63	75	74	76	53	69
166.6667 µg/ml	21.93	58	74	81	74	63	67
55.5556 µg/ml	20.84	61	62	79	74	71	78
55.5556 µg/ml	20.31	75	85	83	76	71	87

Treatment	**Mean of viability clones**	**Number of viable cells (x10E6)**	**Cytotoxicity (% of control)**
Negative control	77.83	14.87	
Negative control	64.67	13.00	
Positive control:			
DMN 1 µl/ml	45.83	6.17	55.70
DMN 1 µl/ml	50.50	7.50	46.20
CGA 108 906 tech.:			
1500.0000 µg/ml	*	*	*
1500.0000 µg/ml	*	*	*
500.0000 µg/ml	72.67	13.93	0.02
500.0000 µg/ml	66.17	13.05	6.35
166.6667 µg/ml	68.33	14.14	nTx
166.6667 µg/ml	69.50	15.24	nTx
55.5556 µg/ml	70.83	14.76	nTx
55.5556 µg/ml	79.50	16.14	nTx

TABLE 20 : 6-TG RESISTANT (MUTANT) CLONES COUNTED
Experiment with metabolic activation

Test number : 983071
Experiment : 1st Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	6-Thioguanine resistant mutant clones counted (per flask)				Mean of the mutant clones counted
Negative control	9	5	9	6	7.25
Negative control	6	3	6	5	5.00
Positive control:					
DMN 1 µl/ml	117	124	123	118	120.50
DMN 1 µl/ml	126	116	112	119	118.25
CGA 108 906 tech.:					
1500.0000 µg/ml	Tx	Tx	Tx	Tx	*
1500.0000 µg/ml	Tx	Tx	Tx	Tx	*
500.0000 µg/ml	4	5	3	6	4.50
500.0000 µg/ml	6	4	2	6	4.50
166.6667 µg/ml	1	2	2	4	2.25
166.6667 µg/ml	6	6	5	4	5.25
55.5556 µg/ml	9	10	7	4	7.50
55.5556 µg/ml	1	3	3	4	2.75

TABLE 21 : CYTOTOXICITY DETERMINED AFTER TREATMENT
Experiment without metabolic activation

Test number : 983071
Experiment : 1st Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	**Cell number after treatment (x10E6)**	**Survival clones after treatment (per well)**					
Negative control	20.96	105	99	96	99	96	94
Negative control	20.13	92	92	94	94	96	94
Positive control:							
EMS 0.3 µl/ml	18.83	78	96	84	85	85	65
EMS 0.3 µl/ml	18.98	85	94	96	85	75	94
CGA 108 906 tech.:							
1000.0000 µg/ml	13.94	58	59	78	69	68	69
1000.0000 µg/ml	13.25	78	81	58	62	70	83
333.3333 µg/ml	20.22	93	99	96	98	94	93
333.3333 µg/ml	20.86	96	97	95	96	94	90
111.1111 µg/ml	20.29	98	95	94	92	98	96
111.1111 µg/ml	20.71	94	94	96	90	96	95
37.0370 µg/ml	20.62	99	99	95	96	92	94
37.0370 µg/ml	20.78	94	96	95	95	95	91

Treatment	**Mean of survival clones**	**Number of viable cells (x10E6)**	**Acute cytotoxicity (% of control)**
Negative control	98.17	20.57	
Negative control	93.67	18.85	
Positive control:			
EMS 0.3 µl/ml	82.17	15.47	21.51
EMS 0.3 µl/ml	88.17	16.73	15.12
CGA 108 906 tech.:			
1000.0000 µg/ml	66.83	9.32	52.74
1000.0000 µg/ml	72.00	9.54	51.59
333.3333 µg/ml	95.50	19.31	2.05
333.3333 µg/ml	94.67	19.75	nTx
111.1111 µg/ml	95.50	19.38	1.69
111.1111 µg/ml	94.17	19.50	1.06
37.0370 µg/ml	95.83	19.76	nTx
37.0370 µg/ml	94.33	19.61	0.54

TABLE 22 : NUMBER OF VIABLE CELLS AT SUBCULTURE
Experiment without metabolic activation

Test number : 983071
Experiment : 1st Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number at subculture (x10E6)	% growth of control
Negative control	16.59	
Negative control	16.38	
Positive control:		
EMS 0.3 µl/ml	11.49	69.72
EMS 0.3 µl/ml	11.10	67.32
<u>CGA 108 906 tech.:</u>		
1000.0000 µg/ml	13.61	82.56
1000.0000 µg/ml	11.37	68.95
333.3333 µg/ml	15.21	92.28
333.3333 µg/ml	13.58	82.39
111.1111 µg/ml	14.44	87.61
111.1111 µg/ml	14.87	90.20
37.0370 µg/ml	14.09	85.46
37.0370 µg/ml	17.50	106.16

TABLE 23 : CYTOTOXICITY DETERMINED AFTER EXPRESSION
Experiment without metabolic activation

Test number : 983071
Experiment : 1st Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	**Cell number after expression (x10E6)**	**Viability clones after expression (per well)**					
Negative control	19.65	46	44	34	47	41	41
Negative control	17.96	40	52	33	48	50	30
Positive control:							
EMS 0.3 µl/ml	15.79	40	46	48	42	47	46
EMS 0.3 µl/ml	16.50	47	62	52	51	53	54
CGA 108 906 tech.:							
1000.0000 µg/ml	17.32	52	49	46	41	55	46
1000.0000 µg/ml	16.40	60	54	40	54	56	46
333.3333 µg/ml	17.84	64	61	51	53	51	53
333.3333 µg/ml	18.05	51	53	62	47	45	50
111.1111 µg/ml	18.34	45	45	35	43	47	33
111.1111 µg/ml	17.32	44	37	41	56	31	47
37.0370 µg/ml	17.68	49	47	44	40	54	38
37.0370 µg/ml	15.80	52	61	60	67	61	54

Treatment	**Mean of viability clones**	**Number of viable cells (x10E6)**	**Cytotoxicity (% of control)**
Negative control	42.17	8.28	
Negative control	42.17	7.58	
Positive control:			
EMS 0.3 µl/ml	44.83	7.08	10.71
EMS 0.3 µl/ml	53.17	8.77	nTx
CGA 108 906 tech.:			
1000.0000 µg/ml	48.17	8.34	nTx
1000.0000 µg/ml	51.67	8.47	nTx
333.3333 µg/ml	55.50	9.90	nTx
333.3333 µg/ml	51.33	9.26	nTx
111.1111 µg/ml	41.33	7.58	4.38
111.1111 µg/ml	42.67	7.39	6.83
37.0370 µg/ml	45.33	8.01	nTx
37.0370 µg/ml	59.17	9.35	nTx

TABLE 24 : 6-TG RESISTANT (MUTANT) CLONES COUNTED
Experiment without metabolic activation

Test number : 983071
Experiment : 1st Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	6-Thioguanine resistant mutant clones counted (per flask)				Mean of the mutant clones counted
Negative control	1	2	4	2	2.25
Negative control	1	4	2	2	2.25
Positive control:					
EMS 0.3 µl/ml	966	954	963	958	960.25
EMS 0.3 µl/ml	982	988	979	984	983.25
<u>CGA 108 906 tech.:</u>					
1000.0000 µg/ml	1	1	3	1	1.50
1000.0000 µg/ml	1	3	2	5	2.75
333.3333 µg/ml	2	5	1	3	2.75
333.3333 µg/ml	1	1	2	2	1.50
111.1111 µg/ml	2	5	3	4	3.50
111.1111 µg/ml	2	3	3	3	2.75
37.0370 µg/ml	1	2	1	1	1.25
37.0370 µg/ml	3	3	1	1	2.00

TABLE 25 : CYTOTOXICITY DETERMINED AFTER TREATMENT
Experiment with metabolic activation

Test number : 983071
Experiment : 2nd Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number after treat-ment (x10E6)	Survival clones after treatment (per well)					
Negative control	7.00	42	39	36	38	32	31
Negative control	5.91	33	36	43	35	47	46
Positive control:							
DMN 1 µl/ml	5.86	31	26	35	16	29	31
DMN 1 µl/ml	6.51	40	44	38	38	37	49
CGA 108 906 tech.:							
1350.0000 µg/ml	1.78	0	1	0	0	0	1
1350.0000 µg/ml	2.59	0	3	2	1	1	0
900.0000 µg/ml	4.21	47	39	58	40	45	40
900.0000 µg/ml	4.98	39	31	34	40	41	44
600.0000 µg/ml	5.38	49	63	47	53	46	60
600.0000 µg/ml	5.78	57	56	64	55	59	55
400.0000 µg/ml	5.89	60	55	66	48	58	60
400.0000 µg/ml	6.06	60	54	47	49	66	59

Treatment	Mean of survival clones	Number of viable cells (x10E6)	Acute cyto-toxicity (% of control)
Negative control	36.33	2.54	
Negative control	40.00	2.37	
Positive control:			
DMN 1 µl/ml	28.00	1.64	33.10
DMN 1 µl/ml	41.00	2.67	nTx
CGA 108 906 tech.:			
1350.0000 µg/ml	0.33	0.01	99.76
1350.0000 µg/ml	1.17	0.03	98.77
900.0000 µg/ml	44.83	1.89	23.11
900.0000 µg/ml	38.17	1.90	22.52
600.0000 µg/ml	53.00	2.85	nTx
600.0000 µg/ml	57.67	3.34	nTx
400.0000 µg/ml	57.83	3.41	nTx
400.0000 µg/ml	55.83	3.39	nTx

TABLE 26 : NUMBER OF VIABLE CELLS AT SUBCULTURE
Experiment with metabolic activation

Test number : 983071
Experiment : 2nd Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number at subculture (x10E6)	% growth of control
Negative control	18.80	
Negative control	19.32	
Positive control:		
DMN 1 µl/ml	17.43	91.42
DMN 1 µl/ml	16.50	86.57
CGA 108 906 tech.:		
1350.0000 µg/ml	0.14	0.72
1350.0000 µg/ml	0.34	1.80
900.0000 µg/ml	17.89	93.87
900.0000 µg/ml	17.54	92.01
600.0000 µg/ml	18.97	99.55
600.0000 µg/ml	16.91	88.72
400.0000 µg/ml	18.31	96.07
400.0000 µg/ml	18.13	95.10

TABLE 27 : CYTOTOXICITY DETERMINED AFTER EXPRESSION
Experiment with metabolic activation

Test number : 983071
Experiment : 2nd Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number after expression (x10E6)	Viability clones after expression (per well)					
Negative control	18.31	77	76	70	66	75	79
Negative control	19.10	85	71	76	74	73	*
Positive control:							
DMN 1 µl/ml	15.35	70	71	79	71	81	75
DMN 1 µl/ml	15.45	66	55	62	67	63	73
CGA 108 906 tech.:							
1350.0000 µg/ml	Tx	Tx	Tx	Tx	Tx	Tx	Tx
1350.0000 µg/ml	Tx	Tx	Tx	Tx	Tx	Tx	Tx
900.0000 µg/ml	22.26	74	82	79	83	81	84
900.0000 µg/ml	26.15	79	78	65	76	79	69
600.0000 µg/ml	21.21	76	65	70	66	75	64
600.0000 µg/ml	19.40	74	83	83	75	82	86
400.0000 µg/ml	18.76	78	77	82	75	76	83
400.0000 µg/ml	18.39	76	84	72	85	73	79

Treatment	Mean of viability clones	Number of viable cells (x10E6)	Cytotoxicity (% of control)
Negative control	73.83	13.52	
Negative control	75.80	14.47	
Positive control:			
DMN 1 µl/ml	74.50	11.44	18.30
DMN 1 µl/ml	64.33	9.94	29.00
CGA 108 906 tech.:			
1350.0000 µg/ml	*	*	*
1350.0000 µg/ml	*	*	*
900.0000 µg/ml	80.50	17.92	nTx
900.0000 µg/ml	74.33	19.44	nTx
600.0000 µg/ml	69.33	14.71	nTx
600.0000 µg/ml	80.50	15.62	nTx
400.0000 µg/ml	78.50	14.72	nTx
400.0000 µg/ml	78.17	14.37	nTx

TABLE 28 : 6-TG RESISTANT (MUTANT) CLONES COUNTED
Experiment with metabolic activation

Test number : 983071
Experiment : 2nd Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	6-Thioguanine resistant mutant clones counted (per flask)				Mean of the mutant clones counted
Negative control	4	3	3	4	3.50
Negative control	3	3	3	4	3.25
Positive control:					
DMN 1 µl/ml	124	119	132	129	126.00
DMN 1 µl/ml	121	137	129	117	126.00
<u>**CGA 108 906 tech.:**</u>					
1350.0000 µg/ml	Tx	Tx	Tx	Tx	*
1350.0000 µg/ml	Tx	Tx	Tx	Tx	*
900.0000 µg/ml	2	2	2	2	2.00
900.0000 µg/ml	4	2	3	2	2.75
600.0000 µg/ml	2	3	2	3	2.50
600.0000 µg/ml	5	4	3	2	3.50
400.0000 µg/ml	9	5	9	7	7.50
400.0000 µg/ml	6	6	7	5	6.00

TABLE 29 : CYTOTOXICITY DETERMINED AFTER TREATMENT
Experiment without metabolic activation

Test number : 983071
Experiment : 2nd Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number after treatment (x10E6)	Survival clones after treatment (per well)					
Negative control	10.16	92	86	97	88	91	89
Negative control	10.32	96	92	88	89	94	84
Positive control:							
EMS 0.3 µl/ml	9.49	69	62	59	61	63	62
EMS 0.3 µl/ml	10.77	54	52	63	64	59	51
CGA 108 906 tech.:							
1200.0000 µg/ml	2.66	21	21	22	28	20	36
1200.0000 µg/ml	3.71	29	19	35	24	25	25
1100.0000 µg/ml	5.65	51	44	32	40	41	36
1100.0000 µg/ml	5.37	50	56	50	45	50	46
1000.0000 µg/ml	7.64	54	56	44	67	42	47
1000.0000 µg/ml	6.73	64	71	69	70	67	67
900.0000 µg/ml	5.84	53	57	50	48	55	55
900.0000 µg/ml	5.62	47	51	50	52	46	52

Treatment	Mean of survival clones	Number of viable cells (x10E6)	Acute cytotoxicity (% of control)
Negative control	90.50	9.19	
Negative control	90.50	9.34	
Positive control:			
EMS 0.3 µl/ml	62.67	5.95	35.81
EMS 0.3 µl/ml	57.17	6.16	33.53
CGA 108 906 tech.:			
1200.0000 µg/ml	24.67	0.66	92.92
1200.0000 µg/ml	26.17	0.97	89.52
1100.0000 µg/ml	40.67	2.30	75.18
1100.0000 µg/ml	49.50	2.66	71.30
1000.0000 µg/ml	51.67	3.95	57.42
1000.0000 µg/ml	68.00	4.57	50.63
900.0000 µg/ml	53.00	3.10	66.58
900.0000 µg/ml	49.67	2.79	69.87

TABLE 30 : NUMBER OF VIABLE CELLS AT SUBCULTURE
Experiment without metabolic activation

Test number : 983071
Experiment : 2nd Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number at subculture (x10E6)	% growth of control
Negative control	18.63	
Negative control	19.35	
Positive control:		
EMS 0.3 µl/ml	12.17	64.10
EMS 0.3 µl/ml	10.96	57.70
CGA 108 906 tech.:		
1200.0000 µg/ml	3.34	17.60
1200.0000 µg/ml	3.35	17.63
1100.0000 µg/ml	11.47	60.38
1100.0000 µg/ml	8.59	45.23
1000.0000 µg/ml	15.78	83.07
1000.0000 µg/ml	16.28	85.73
900.0000 µg/ml	10.11	53.23
900.0000 µg/ml	9.88	52.01

TABLE 31 : CYTOTOXICITY DETERMINED AFTER EXPRESSION
Experiment without metabolic activation

Test number : 983071
Experiment : 2nd Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	Cell number after expression (x10E6)	Viability clones after expression (per well)					
Negative control	21.07	56	46	41	45	50	48
Negative control	20.25	56	47	46	50	52	55
Positive control:							
EMS 0.3 µl/ml	14.84	50	44	54	44	51	50
EMS 0.3 µl/ml	15.99	49	56	38	39	52	41
CGA 108 906 tech.:							
1200.0000 µg/ml	16.84	57	53	54	48	55	56
1200.0000 µg/ml	19.49	69	72	64	65	68	64
1100.0000 µg/ml	11.66	37	39	35	28	33	29
1100.0000 µg/ml	10.37	27	28	31	30	34	31
1000.0000 µg/ml	11.14	33	37	38	30	26	30
1000.0000 µg/ml	12.04	35	36	30	29	31	30
900.0000 µg/ml	20.72	66	61	60	62	63	57
900.0000 µg/ml	20.63	50	57	54	57	45	57

Treatment	Mean of viability clones	Number of viable cells (x10E6)	Cytotoxicity (% of control)
Negative control	47.67	10.04	
Negative control	51.00	10.33	
Positive control:			
EMS 0.3 µl/ml	48.83	7.25	28.83
EMS 0.3 µl/ml	45.83	7.33	28.04
CGA 108 906 tech.:			
1200.0000 µg/ml	53.83	9.07	10.99
1200.0000 µg/ml	67.00	13.06	nTx
1100.0000 µg/ml	33.50	3.90	61.66
1100.0000 µg/ml	30.17	3.13	69.29
1000.0000 µg/ml	32.33	3.60	64.65
1000.0000 µg/ml	31.83	3.83	62.37
900.0000 µg/ml	61.50	12.74	nTx
900.0000 µg/ml	53.33	11.00	nTx

TABLE 32 : 6-TG RESISTANT (MUTANT) CLONES COUNTED
Experiment without metabolic activation

Test number : 983071
Experiment : 2nd Confirmatory
Test substance : CGA 108 906 tech.
Batch : KI-5240/3

Treatment	6-Thioguanine resistant mutant clones counted (per flask)				Mean of the mutant clones counted
Negative control	4	6	4	6	5.00
Negative control	2	4	3	3	3.00
Positive control:					
EMS 0.3 µl/ml	824	839	856	848	841.75
EMS 0.3 µl/ml	932	964	961	959	954.00
CGA 108 906 tech.:					
1200.0000 µg/ml	2	5	7	8	5.50
1200.0000 µg/ml	3	6	3	3	3.75
1100.0000 µg/ml	4	5	3	5	4.25
1100.0000 µg/ml	5	3	5	4	4.25
1000.0000 µg/ml	2	3	2	4	2.75
1000.0000 µg/ml	3	2	2	2	2.25
900.0000 µg/ml	3	4	5	4	4.00
900.0000 µg/ml	4	3	2	2	2.75

REPORT OF ANALYTICAL DETERMINATIONS

(7 pages)

CGA 108906 tech. Method-No.: A98060 1/3

Determination of CGA 108906 tech. in aqueous solutions

Objective

Determination of the content of CGA 108906 tech. in aqueous solutions by HPLC.

Test Facility

Novartis Crop Protection AG, Toxicology/Cell Biology, CH-4002 Basel.

Principal Scientist:
Date/Signature: 13.11.98

Principal Investigator:
Date/Signature: 13.11.98

Abstract:

The sample is diluted with acetonitrile and analysed by HPLC (Nucleosil C18, 5 µm, 125 x 4.6mm; acetonitrile/ 0.1% aqueous o-phosphoric acid) with UV detection at 229 nm.

Test Samples

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

Reference Samples

Two stock solutions of CGA 108906 tech. dissolved in acetonitrile are prepared. These stock solutions are diluted with acetonitrile to yield final concentrations in the range of the test samples (R1, R2).

HPLC Conditions

Column:	Nucleosil C18, 5 µm, 250x4.6 mm	
Mobile phase:	Acetonitrile/0.1 % aqueous o-phosphoric acid	
Gradient:	0 min..	20% acetonitrile
	10 min.:	30% acetonitrile
Temperature:	Ambient	
Flow rate:	1.5 ml/min	
Wavelength:	229 nm	
Injection volume:	20 µl	
Retention time:	approx. 6.0 min	

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 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

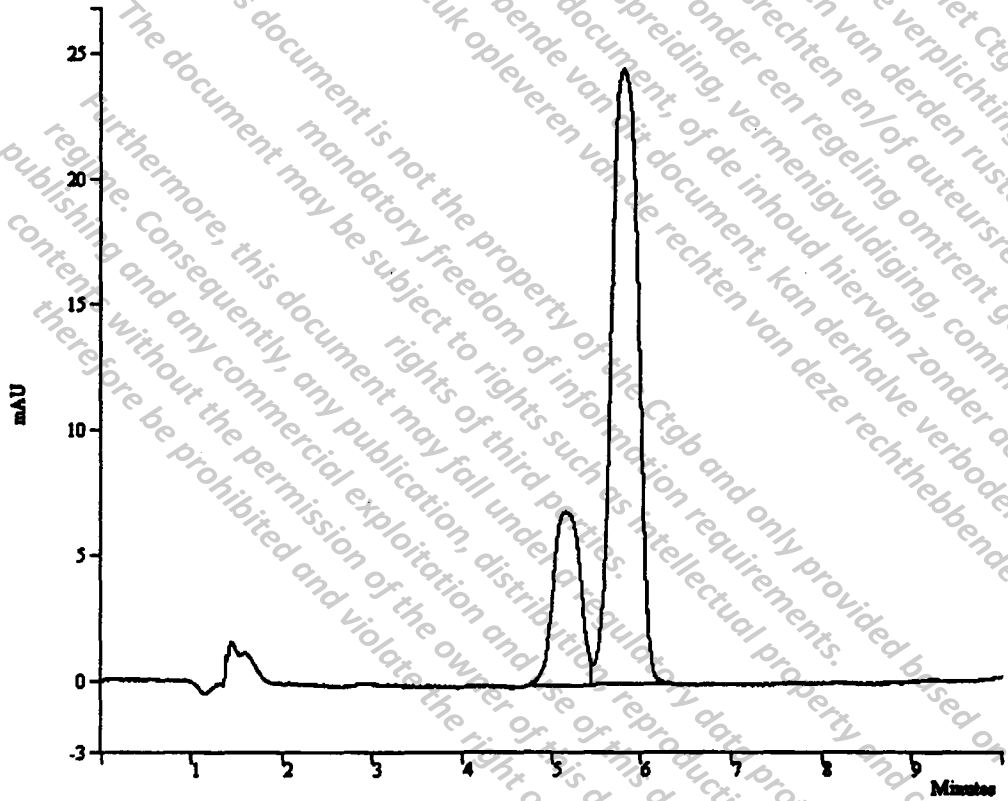
A typical chromatogram of a test sample is shown in Figure 1. The appearance of the chromatograms might depend on actual conditions at a particular time (vehicle, solvents, column, environment).

CGA 108906 tech. Method-No.: A98060 3/3

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 No.: KI-5240/3) in acetonitrile



Novartis Crop Protection AG

ANALYSIS DATA

Test No. : 983071 QA requested : X yes no
Repetition : Original

Test System : GENE MUTATION TEST WITH CHINESE HAMSTER CELLS V79

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

Test Substance: CGA 108 906 tech.
Batch No. : KI-5240/3 Purity % : 99 %
Vehicle : Bidistilled water Storage conditions : - 20°C
Date of Preparation: 15.09.1998

Performed by (Name): [redacted]

Study Director: Mr. [redacted], R-1058.3.42, Tel. [redacted]

[redacted] (Visa)

November 10, 1998 (Date) [redacted] (Signature)

Delivered to Cell Biology: [redacted]

Date: 10.11.98 Name: Zellbiologie R-1058.340 © [redacted] Signature: [redacted]

	Data from Genetic Toxicology		Data from Cell Biology	
	Samples Nominal values (µg/ml)	Vol. (ml)	Determined values (µg/ml)	% of Nominal values
	20'000	5	22011 / 21911	110 / 110
	370.4	5	369,8 / 372,7	99,8 / 100,6

Analysis method: A 98060

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

Performed by: Principal Scientist [redacted]

13.11.98 (Date) [redacted] (Signature)

Approved by: Principal Investigator Dr. [redacted]

12.11.98 (Date) [redacted] (Signature)

(Raw data will be stored in the archives of Genetic Toxicology, Novartis Crop Protection AG, Basel, Switzerland)

Novartis Crop Protection AG

ANALYSIS DATA

Test No. : **983071** **QA requested** : **X** yes no
Repetition : **1 st Confirmatory**

Test System : **GENE MUTATION TEST WITH CHINESE HAMSTER CELLS V79**

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

Test Substance: CGA 108 906 tech
Batch No. : **KI-5240/3** **Purity %** : **99 %**
Vehicle : **Bidistilled water** **Storage conditions** : **- 20°C**
Date of Preparation: 22.09.1998

Performed by (Name): 5.1.2.e Woo

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

(Visa)

10.11.98 5.1.2.e Woo
(Date) (Signature)

Delivered to Cell Biology: 5.1.2.e Woo Zellbiologie

Date: 10.11.98 Name: R - 1058.340 5.1.2.e Woo Signature: 5.1.2.e Woo

	Data from Genetic Toxicology		Data from Cell Biology	
	Samples Nominal values (µg/ml)	Vol. (ml)	Determined values (µg/ml)	% of Nominal values
	15'000	5	14419 / 14407	96.1 / 96.0
	370.4	5	420.1 / 406.7	113 / 110

Analysis method: A 98060

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

Performed by: Principal Scientist 5.1.2.e Woo
13.11.98
(Date) (Signature)

Approved by: Principal Investigator Dr. 5.1.2.e Woo
12.11.98
(Date) (Signature)

[Raw data will be stored in the archives of Genetic Toxicology, Novartis Crop Protection AG, Basel, Switzerland]

Novartis Crop Protection AG

ANALYSIS DATA

Test No. : **983071** **QA requested** : **X yes no**
Repetition : **2 nd Confirmatory**

Test System : **GENE MUTATION TEST WITH CHINESE HAMSTER CELLS V79**

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

Test Substance: CGA 108 906 tech.
Batch No. : **KI-5240/3** **Purity %** : **99 %**
Vehicle : **Bidistilled water** **Storage conditions** : **- 20°C**
Date of Preparation: 13.10.1998

Performed by (Name): **Study Director:**

5.1.2.e Woo

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

(Visa) Vevey, 10. 11. 1998 5.1.2.e Woo
(Date) (Signature)

Delivered to Cell Biology: 5.1.2.e Woo

Zellbiologie 5.1.2.e Woo

Date: 10.11.98 **Name:** R-1058.340 **Signature:** 5.1.2.e Woo

	Data from Genetic Toxicology		Data from Cell Biology	
	Samples Nominal values (µg/ml)	Vol. (ml)	Determined values (µg/ml)	% of Nominal values
	13'500	5	13376 / 13226	99.1 / 98.0
	9'000	5	9104 / 9748	101 / 108

Analysis method: A 98060

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

Performed by: Principal Scientist 5.1.2.e Woo
13.11.98 (Date) (Signature)

Approved by: Principal Investigator Dr. 5.1.2.e Woo
13.11.98 (Date) (Signature)

[Raw data will be stored in the archives of Genetic Toxicology, Novartis Crop Protection AG, Basel, Switzerland]

Distribution

5.1.2.e Woo

2x

1x