STATEMENT

TITLE OF STUDY

Chromosomal Aberration Test of 13F-SFA Using Cultured Mammalian Cells (Study Code K06-1189)

I, the undersigned, hereby declare that this report provides a correct English translation of the final report of the above-mentioned study issued on March 26, 2007.

September 7, 2007

Date

Hita Laboratory
Chemicals Evaluation and Research Institute, Japan

CERI

Receipt No. 837-06-T-5256

STUDY CODE: K06-1189

FINAL REPORT

CHROMOSOMAL ABERRATION TEST OF 13F-SFA USING CULTURED MAMMALIAN CELLS

March 2007

Hita Laboratory
Chemicals Evaluation and Research Institute
Japan

GLP STATEMENT

Hita Laboratory
Chemicals Evaluation and Research Institute, Japan

Sponsor: DAIKIN INDUSTRIES, LTD.
Title: Chromosomal Aberration Test of 13F-SFA Using Cultured Mammalian Cells
Study Code: K06-1189

I, the undersigned, hereby declare that this study was conducted in compliance with "Concerning Standard of the Testing Facilities Conducting the Test Relating to the New Chemical Substances" on Japanese GLP (Notification No. 1121003 of the Pharmaceutical and Food Safety Bureau, MHLW, No. 3 (November 17, 2003) of the Manufacturing Industries Bureau, METI & No. 031121004 of the Environmental Health Department, MOE (November 21, 2003)) and "OECD Principles of Good Laboratory Practice" (November 26, 1997).

I also confirmed that this report accurately reflected the raw data and the test data were valid.

Study Director: Signed in original March 26, 2007

QUALITY ASSURANCE STATEMENT

Hita Laboratory
Chemicals Evaluation and Research Institute, Japan

Sponsor: DAIKIN INDUSTRIES, LTD.
Title: Chromosomal Aberration Test of 13F-SFA Using Cultured Mammalian Cells
Study Code: K06-1189

This study was audited and inspected by Quality Assurance Section of Hita Laboratory, Chemicals Evaluation and Research Institute, Japan. The dates audited and/or inspected and the dates reported these results to the study director and the management are as follows.

Items of Inspections/Audits	Dates of Inspections/Audits	Dates of Inspections/Audits Report
Protocol	December 8, 2006	December 8, 2006
Preparation of Test Substance	December 11, 2006	December 11, 2006
Treatment of Cells	December 11, 2006	December 11, 2006
Reinspection of protocol	December 14, 2006	December 16, 2006
Protocol Amendment	December 20, 2006	December 20, 2006
Protocol Amendment (No. 2)	January 12, 2007	January 12, 2007
Protocol Amendment (No. 3)	February 28, 2007	February 28, 2007
Protocol Amendment (No. 4)	March 2, 2007	March 3, 2007
Reinspecition of Protocol Amendment (No. 4)	March 8, 2007	March 8, 2007
Protocol Amendment (No. 5)	March 9, 2007	March 9, 2007
Protocol Amendment (No. 6)	March 13, 2007	March 13, 2007
Raw Data and Draft Final Report	March 21, 2007	March 21, 2007
Reinspection of Raw Data and Draft Final Report	March 23, 2007	March 23, 2007
Draft Final Report (Second)	March 24, 2007	March 24, 2007
Reinspection of Draft Final Report (Second)	March 26, 2007	March 26, 2007
Final Report	March 26, 2007	March 26, 2007

The inspection result of following phase was reported to the study director and management based on the report of process-based inspection relevant to this study type and timeframe.

Items of Inspections	Dates of Inspections	Dates of Inspections Report
Preparation and Management of Positive Control Substance	November 24, 2006	March 26, 2007
Preparation of Medium and Reagent	December 6 and 7, 2006	March 26, 2007
Preculture of Cells	November 27, 2006	March 26, 2007
Collection of Cells and Preparation of Specimens	December 5, 2006	March 26, 2007

I, the undersigned, hereby declare that this report provides an accurate description of the methods and procedures used in this study and that the reported results accurately reflect obtained raw data.

Head, Quality Assurance Unit: Signed in original March 26, 2007

TABLE OF CONTENTS

Study Code: K06-1189
Test Substance Code: HR6851
Sponsor Code: D-0060

TITLE

Chromosomal Aberration Test of 13F-SFA Using Cultured Mammalian Cells

SPONSOR

DAIKIN INDUSTRIES, LTD.
1-1, Nishihitotsuya, Settsu, Osaka 566-8585, Japan

TESTING FACILITY

Hita Laboratory
Chemicals Evaluation and Research Institute, Japan
822, 3-chome, Ishii-machi, Hita, Oita 877-0061, Japan

PURPOSE OF STUDY

The ability of the test substance to induce chromosomal aberrations was examined by using Chinese hamster lung fibroblasts (CHL/IU cells).

TESTING METHOD

This study was conducted in accordance with "III Mutagenicity Test: Chromosomal Aberration Test Using Cultured Mammalian Cells" prescribed in "Concerning Testing Methods Relating to the New Chemical Substances" on Japanese Test Guideline (Notification No. 1121002 of the Pharmaceutical and Food Safety Bureau, MHLW, No. 2 (November 13, 2003) of the Manufacturing Industries Bureau, METI & No. 031121002 of the Environmental Health Department, MOE (November 21, 2003)).

GLP COMPLIANCE

This study was conducted in compliance with "Concerning Standard of the Testing Facilities Conducting the Test Relating to the New Chemical Substances" on Japanese GLP (Notification No. 1121003 of the Pharmaceutical and Food Safety Bureau, MHLW, No. 3 (November 17, 2003) of the Manufacturing Industries Bureau, METI & No. 031121004 of the Environmental Health Department, MOE (November 21, 2003)) and "OECD Principles of Good Laboratory Practice" (November 26, 1997).

PERIOD OF STUDY

Commencement of Study:	December 7, 2006
Initiation of Experiment (Initiation of Cell Growth Inhibition Test):	December 11, 2006
Completion of Experiment (Completion of Observation of Specimens):	March 12, 2007
Completion of Study:	March 26, 2007

STORAGE AND RETENTION PERIOD OF DATA

The raw data, protocol, protocol amendment, letter of test request, table of test substance information, final report, other record documents and specimens will be stored in the archive of this testing facility for 10 years after the date of receipt of the notification that they are applicable to Article 4, Paragraphs 1 or 2, Article 4-2, Paragraphs 2, 3 or 8, Article 5-4, Paragraph 2, Article 24, Paragraph 2 or Article 25-3, Paragraph 2 of the Japanese Chemical Substances Control Law No. 117 (1973). The sponsor will inform the date of receipt of the notification to this testing facility. After termination of the retention period, any measures taken will be done so with the approval of the sponsor.

The specimens to which the quality will be deteriorated will be retained only for the period when the quality can be secured. The sponsor's consent will be obtained before abandonment.

RETENTION OF ORIGINAL DOCUMENTS

An original protocol, original protocol amendments and an original final report will be retained at the testing facility. The copies of their original that the study director was recognized to be accurate copy were sent to the sponsor.

STUDY DIRECTOR AND PERSONS CONCERNED WITH THE STUDY AND THE OPERATION

Study Director:

Mutagenicity Section, Hita Laboratory

Persons Concerned with The Study and Their Operation:

(Preparation of test substance solution, cell treatment and microscopic observation of specimens)

(Microscopic observation of specimens)

APPROVAL BY AUTHOR OF FINAL REPORT

Study Director: Signed in original March 26, 2007

SUMMARY

The ability of 13F-SFA to induce chromosomal aberrations was investigated by using Chinese hamster lung fibroblasts (CHL/IU cells).

Based on the result of cell growth inhibition test, the doses of the test substance in the chromosomal aberration test were set at 26.6, 37.2, 52.1, 72.9, 102, 143, 200 and 280 μg/mL in short-term treatments without and with S9 mix and at 19.0, 26.6, 37.2, 52.1, 72.9, 102, 143 and 200 μg/mL in 24 hours continuous treatment.

In the chromosomal aberration test, the doses for observation of specimens were selected at 52.1, 72.9 and 102 μg/mL for the short-term treatment without S9 mix and at 72.9, 102 and 143 μg/mL for the short-term treatment with S9 mix and at 37.2, 52.1, 72.9 and 102 μg/mL for 24 hours continuous treatment. In the observation, the frequencies of cells with structural aberrations and numerical aberration cells were scored.

As a result of observation of specimens, the frequencies of numerical aberration cells were below 5% at all observation doses of the test substance in each treatment, therefore, numerical aberration was judged to be negative. However, in 24 hours continuous treatment, the frequency of cells with structural aberration was over 10% and the frequencies were recognized as a dose-related increase. Therefore, structural aberration was judged to be positive.

On the other hand, the frequencies of cells with structural aberrations or numerical aberration cells in the negative control treated with dehydrated acetone showed below 5%, and the frequencies of cells with structural aberrations in the positive controls treated with mitomycin C or cyclophosphamide monohydrate, showed above 20%, indicating the proper performance of the present study.

It was concluded that 13F-SFA did not induce numerical aberration but it induced structural aberration under the present test conditions.

MATERIALS AND METHODS

1. TEST SUBSTANCE AND POSITIVE CONTROL SUBSTANCES

1.1 Test Substance (Information Provided by the Sponsor)

1) Name

3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl=acrylate

Other name: 13F-SFA

CAS No.: 17527-29-6

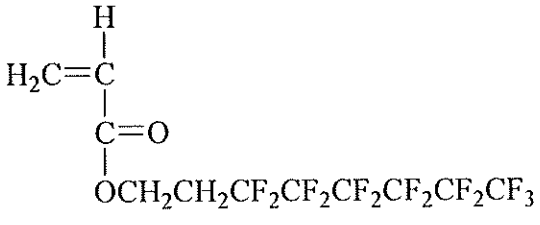
2) Lot No.

6X002

3) Supplier

DAIKIN INDUSTRIES, LTD.

4) Structural formula



(Molecular formula: $C_{11}H_7F_{13}O_2$)

5) Purity

99.7%

6) Names and concentrations of impurities

Unknown composition	0.3%

7) Physicochemical properties

Appearance at ordinary temperature:	colorless transparent liquid
Molecular weight:	418.15
Stability:	—
Melting point:	—
Boiling point:	78°C (8 mmHg)
Vapor pressure:	—
Partition coefficient (1- octanol/water):	—
Hydrolysis:	hydrolyzes $CH_2=CHCOOCH_2CH_2C_6F_{13} \rightarrow C_6F_{13}CH_2CH_2OH+CH_2=CHCOOH$
Solubility:	—

Degree of solubility

Water:	insoluble
DMSO:	<50.0 mg/mL (degree of solubility to dehydrated DMSO was measured at the testing facility)
Acetone:	≥418 mg/mL (degree of solubility to dehydrated acetone was measured at the testing facility)
Other:	—

8) Storage conditions

Stored in light-shading at room temperature (decicator No. 2 in the test substance storage room, permissible limit of temperature: 10 to 30°C).

9) Precautions

Gloves, a mask, a head cap and a lab coat were worn.

1.2 Positive Control Substances

1) Mitomycin C (MMC)

Manufacturer:	Kyowa Hakko Kogyo Co., Ltd.
Lot No.:	480AEL
Appearance:	royal purple powder
Content:	99%
Grade:	for injection

2) Cyclophosphamide monohydrate (CPA)

Manufacturer:	Wako Pure Chemical Industries, Ltd.
Lot No.:	PKQ7031
Appearance:	white crystals or crystalline powder
Content:	99.0%
Grade:	for biochemistry

3) Storage conditions

MMC was stored at room temperature (cabinet No. 2 in the test substance storage room, the tolerance temperature: 10 to 30°C) and CPA in a cold dark place (refrigerator No.13 in the test substance storage room, the tolerance temperature: 1 to 10°C).

4) Precautions

Gloves, a mask, a head cap and a lab coat were worn.

2. CELLS

2.1 Cell Line and Reason for Selection

Chinese hamster lung fibroblasts (CHL/IU cells) were supplied by Health Science Research Resources Bank, Japan Health Sciences Foundation on April 17, 2002. The modal number of chromosomes was 25 per cell. The doubling time was about 15 hours. It was confirmed in the testing facility that the cells were mycoplasma free and the frequencies of cells with structural aberration and the numerical aberration cells were below 5%.

CHL/IU cells have been recommended the availability on *in vitro* chromosome aberration test "Concerning Testing Methods Relating to the New Chemical Substances" on Japanese Test Guideline.

2.2 Storage

Cells were suspended in medium [Eagle's minimum essential medium (Nissui Pharmaceutical Co., Ltd.) and 10 vol% heat-inactivated newborn calf serum (NBCS, Sanko Junyaku Co., Ltd.)] including 10 vol% dimethyl sulfoxide (DMSO) and were frozen in liquid nitrogen.

2.3 Culture Condition

Cells were cultured in a CO_2 incubator (MCO-345, SANYO Electric Co., Ltd. and Model 530, Wakenyaku Co., Ltd.), which was set at 37°C and 5% CO_2 under humid condition.

2.4 Subculture

Cells were subcultured in 90-mm diameter Petri dishes (Nunc A/S) twice a week. Passage number of cells was at 6 for the cell growth inhibition test and 9 for the chromosomal aberration test and 12 for the confirmation test after the receipt.

3. MEDIUM AND S9 MIX

3.1 Medium

L-glutamine (final concentration: 0.292 g/L) and sodium hydrogen carbonate (final concentration: approximately 1.85 g/L) were added to Eagle's minimum essential medium (Lot No. 54860611, Nissui Pharmaceutical Co., Ltd.). This medium was then supplemented with 10 vol% heat-inactivated NBCS (Lot No. 27020859, Sanko Junyaku Co., Ltd.).

3.2 S9 Mix

1) Rat liver S9

S9 (Lot No. 06090106, manufactured on September 1, 2006, protein content: 20.2 mg/mL, Oriental Yeast Co., Ltd.), which was prepared from livers of 7-week-old male SD rat (body weight of rats: 215.1±10.7 g) administered a

combination of phenobarbital and 5,6-benzoflavone was used. S9 was frozen and preserved in an ultra-deep freezer (MDF-U481ATR, SANYO Electric Co., Ltd., the tolerance temperature: below -80°C) until use. S9 was used within six months after the day of manufacturing.

2) Composition of S9 mix

One milliliter of S9 mix consisted of 0.3 mL S9, 5 μmol $MgCl_2$, 33 μmol KCl, 5 μmol glucose-6-phosphate, 4 μmol NADP and 4 μmol HEPES (pH 7.2) and S9 mix was prepared just prior to use and was stored in ice until use.

4. CELL PRE-CULTURE

A 60-mm diameter plastic dish (Asahi Techno Glass Corporation) was used for cell culture. Five milliliters of a cell suspension of 5.0×10^3 cells/mL were seeded into a dish and were cultured continuously for 3 days.

5. PREPARATION OF TEST SUBSTANCE SOLUTION AND POSITIVE CONTROL SUBSTANCE SOLUTIONS

5.1 Preparation of Test Substance Solution

1) Solvent

Acetone (Lot No. EWJ5080, 100.0% in purity, special grade, Wako Pure Chemical Industries, Ltd.) dehydrated with Molecular Sieves 5A 1/8 (Lot No. CEQ7072, Wako Pure Chemical Industries, Ltd.) was used.

2) Reason for selection of solvent

Because the test substance was hydrolyzed (the information from sponsor), degree of solubility in water was not confirmed. Though the test substance was insoluble at 50.0 mg/mL in dehydrated DMSO, the test substance was dissolved at 418 mg/mL in dehydrated acetone. The test substance solution at 418 mg/mL in dehydrated acetone was neither any change in color nor exothermic at room temperature within 2 hours after preparation. Therefore, dehydrated acetone was preferably selected as a solvent in this study.

3) Preparation method

After the test substance was weighed, dehydrated acetone was added to the test substance to make an original solution using a tube mixer. A required concentration series of the test substance solutions was prepared by dilution with the same solvent and prepared to 100 times concentrations of the test substance in the medium. The test substance solutions were prepared under the yellow lamp. It was prepared without correcting purity of the test substance because the purity of the test substance was above 95%.

4) Preparation time and storage

The test substance solutions were prepared immediately before use, stored in ice under the yellow lamp and used within 1 hour after preparation.

5.2 Preparation of Positive Control Substance Solutions

1) Preparation method and storage

MMC and CPA were dissolved in distilled water at 0.01 mg/mL and 1 mg/mL, respectively. The positive control substance solutions were frozen in an ultra-deep freezer (MDF-U481ATR, SANYO Electric Co., Ltd., the tolerance temperature: below -80°C).

2) Preparation time and expiry in use

The positive control substance solutions were thawed at the time of use and used within 1.5 hours. The stock solutions were used within 6 months after preparation.

6. TEST PROCEDURE

6.1 Cell Growth Inhibition Test

1) Procedure

For the short-term treatment without S9 mix, the medium was removed from a pre-culture, and the cells were treated for 6 hours in well mixed medium containing 30 μL of the test substance solution or the solvent and 3 mL of the fresh medium. For the short-term treatment with S9 mix, the medium was removed from a pre-culture, and the cells were treated for 6 hours in well mixed medium consisting of 0.5 mL of S9 mix and 30 μL of the test substance solution or the solvent and 2.5 mL of the fresh medium. After treatment, the medium was removed, and the cells were rinsed 3 times with 2 mL of Dulbecco's physiological phosphate buffered solution without Ca^{2+} and Mg^{2+}. Cells were then cultured for another 18 hours in 5 mL of fresh medium.

For the continuous treatment, the medium was removed from a pre-culture, and the cells were treated for 24 hours with well mixed medium containing 50 μL of the test substance solution or the solvent and 5 mL of the fresh medium.

In the short-term and the continuous treatments, 50 μL of a 10 μg/mL demecolcine solution was added to each dish at 2 hours before the end of the culture.

At the start and the end of the treatment and at the end of the culture, precipitation of the test substance, the color change of the medium and the corrosion of the dish were observed macroscopically.

At the end of the culture, a cell suspension was prepared to collect from each

dish by a treatment with 0.25 w/v% trypsin of 2 mL. After 200 μL of the cell suspension was diluted with 10 mL of Cell Pack (Sysmex Corporation), the number of the cells was measured using a Microcell counter (CDA-500, Sysmex Corporation), and the cell growth rate and the 50% cell growth inhibition concentration (IC_{50}) was calculated. The IC_{50} was obtained from a linear line drawn between 2 plots; the one being greater and the other lower than, and both closest to 50% of the cell growth rate.

Remained cells were then collected by a centrifugation at 1000 rpm (185×g) for 5 minutes and were treated hypotonically with 3 mL of 0.075 mol/L KCl at 37°C for 15 minutes. Following the hypotonic treatment, the cells were pre-fixed once with approximately 0.3 mL of a fixative solution (methanol : acetic acid = 3 : 1), and were completely fixed twice with 3 mL of fixative solution. Then, the cell suspension was prepared with a fixative solution, two drops of the suspension were placed on a slide glass, and stained about 15 minutes with 2 vol% Giemsa solution in 1/15 mol/L phosphate buffer solution (pH6.8). One specimen was prepared per dose.

2) Dose levels

In each treatment method, the highest dose was set at 4180 μg/mL as equivalent to 10 mmol/L, as the maximum dose in the case of no cytotoxicity on the guideline, and 8 lower doses, 16.3, 32.7, 65.3, 131, 261, 523, 1050 and 2090 μg/mL were set based on a geometric progression of 2. Duplicate dishes were used for each dose.

3) Observation and scoring

Specimens were observed to check the presence or absence of mitotic metaphases, and the frequency of the cells with chromosomal aberration was calculated by observed 50 metaphases per dose at which the dose setting of chromosomal aberration test was considered to be referred.

(1) Structural aberration

The number of metaphases with structural aberrations excluding gaps was recorded. Gaps were defined as an achromatic region smaller than the width of one chromatid.

(2) Numerical aberration

The number of polyploid showing triploid or more was scored.

6.2 Chromosomal Aberration Test

1) Procedure

Chromosomal aberration test was carried out using the same procedure as that of the cell growth inhibition test, with the following positive controls. Four

specimens per dose (2 specimens per dish) were prepared.

Treatment method		Substance	Dose
Short-term treatment	without S9 mix	MMC	0.1 μg/mL
	with S9 mix	CPA	6 μg/mL
24 hous continuous treatment		MMC	0.05 μg/mL

In the positive control, each dish was added with 30 μL of a 0.01 mg/mL MMC solution and 18 μL of a 1 mg/mL CPA solution for the short-term treatments without and with S9 mix, respectively, and 25 μL of a 0.01 mg/mL MMC solution for the continuous treatment.

2) Dose levels

From the results of the cell growth inhibition test, a cytotoxicity that the cell growth rate was below 50% was obtained in each treatment. However, the decrease of the cell growth rate was inhibited at the range of high doses and the metaphases cells required to evaluate were observed at 4180 μg/mL as equivalent to 10 mmol/L. This phenomenon was seen at the dose that precipitation of the test substance was observed. Then, it was inferred that the test substance had not been dispersed in the medium of the high doses and the decrease of the cell growth rate was inhibited.

Therefore, the highest dose of the test substance was selected at the dose that was over IC_{50}. In the short-term treatments without and with S9 mix, IC_{50} were calculated at 85 and 100 μg/mL, respectively, then the highest dose was selected at 280 μg/mL. In 24 hours continuous treatment, IC_{50} were calculated at 60 μg/mL, then the highest dose was selected at 200 μg/mL. The following 8 doses were set based on a geometric progression of 1.4 in each treatment.

Treatment method		Setting dose of the test substance
Short-term treatment	Without S9 mix	26.6, 37.2, 52.1, 72.9, 102, 143, 200 and 280 μg/mL
	With S9 mix	26.6, 37.2, 52.1, 72.9, 102, 143, 200 and 280 μg/mL
24 hours continuous treatment		19.0, 26.6, 37.2, 52.1, 72.9, 102, 143 and 200 μg/mL

Duplicate dishes were used for each dose.

3) Observation

(1) Dose for observation

All specimens of the negative and the positive controls set as the control groups were observed.

The observation doses of the test substance were selected the consecutive doses of three stages. The selection doses and the selection reason are shown below.

In each treatment, because a cytotoxicity that the cell growth rate was below 50% was obtained, the highest dose for observation was selected at the lowest

dose that the cell growth rate was below 50%. In the short-term treatment without S9 mix, the lowest dose that the cell growth rate was below 50% was 102 μg/mL, therefore, the doses for observation were selected at 52.1, 72.9 and 102 μg/mL. In the short-term treatment with S9 mix, the lowest dose that the cell growth rate was below 50% was 143 μg/mL, therefore, the doses for observation were selected at 72.9, 102 and 143 μg/mL. In 24 hours continuous treatment, the lowest dose that the cell growth rate was below 50% was 72.9 μg/mL, therefore, the doses for observation were selected at 37.2, 52.1 and 72.9 μg/mL.

After the selection of the observation doses, randomly slide numbers were allocated to all observed specimens. All specimens were observed in a blinded manner.

As a result of observation of the above doses, the frequency of cells with structural aberrations was 7.5% at 72.9 μg/mL as the maximum observation dose in 24 hours continuous treatment and the induction of the structural aberration was doubted. It was possible to observe the chromosome at 102 μg/mL that was the higher dose than 72.9 μg/mL. Therefore, additional observation was carried out at 102 μg/mL. Specimens of additional observation were allocated slide code numbers and observed.

(2) Structural Aberration

Two hundred metaphase cells per dose (50 cells per specimen) containing 25±2 chromosomes were scored using a microscope. The total number of cells with structural aberrations and the number of aberrant cells in each aberration category were recorded. Gaps were defined as an achromatic region smaller than the width of one chromatid and were recorded separately from the structural aberrations.

(3) Numerical Aberration

The numbers of polyploid (cell with 38 or more chromosomes) cells among 200 metaphase cells per dose (50 cells per specimen) observed were recorded.

6.3 Confirmation Test

In the chromosomal aberration test for the short-term treatment without S9 mix, the frequency of cells with structural aberrations was 27.0% at 72.9 μg/mL and the induction of the structural aberration was doubted. However, the frequencies were not recognized as a dose-related increase, therefore, the confirmation test for the short-term treatment without S9 mix was carried out. The highest dose was selected at 150 μg/mL and 7 lower doses of 41.9, 50.2, 60.3, 72.3, 86.8, 104 and 125 μg/mL were set based on a geometric progression of 1.2. As a result of

confirmation test, because a cytotoxicity that the cell growth rate was below 50% was obtained, the highest dose for observation was selected at the lowest dose that the cell growth rate was below 50% and the observation doses were selected the consecutive doses of three stages. The lowest dose that the cell growth rate was below 50% was 86.8 μg/mL, therefore, the doses for observation were selected at 60.3, 72.3 and 86.8 μg/mL. The test procedures and the observation method in the confirmation test were carried out in accordance with the chromosomal aberration test.

7. JUDGEMENT CRITERIA OF RESULTS

The findings were judged to be positive when the frequencies of cells with structural aberrations showed 10% or more with a dose-related increase or the frequencies of cells with structural aberrations showed 5% or more both in the chromosomal aberration test and the confirmation test. The other cases were judged to be negative. No statistical analyses were used. The frequency of numerical aberration cells was judged according to the same criteria as that of the structural aberration. In the treatment method that chromosomal aberrations were shown 5% or more, D_{20} value indicating a concentration which will induce chromosomal aberration of 20% was calculated.

8. VALIDITY OF TEST

This study was regarded as valid as follows: 1) the frequencies of cells with chromosomal aberrations did not fluctuate markedly between two culture dishes, 2) the frequencies in the negative control showed below 5%, and 3) the frequencies of cells with structural aberrations excluding gaps in the positive controls showed 20% or more.

FACTORS AFFECTED RELIABILITY OF TEST

There were no factors, which might affect the reliability of the test.

TEST RESULTS

1. CELL GROWTH INHIBITION TEST (Table 1 and Fig. 1)

The IC_{50} were calculated at 85 μg/mL in the short-term treatment without S9 mix, at 100 μg/mL in the short-term treatment with S9 mix and at 60 μg/mL in 24 hours continuous treatment.

At the start of the treatment, precipitation of the test substance was observed at 131 μg/mL or more in each treatment. At the end of the treatment, precipitation of the test substance was observed at 131 μg/mL or more in the short-term treatments without and with S9 mix and at 261 μg/mL or more in 24 hours continuous treatment. At the end of the culture, precipitation of the test substance was observed at 1050 μg/mL or more in the short-term treatment without S9 mix. Color change of the medium and the corrosion of the culture dish were not observed in all doses.

Though the frequencies of the numerical aberration cells were below 5% at the observation doses of the test substance in each treatment, the maximum frequencies of cells with structural aberration were 10.0% in the short-term treatment without S9 mix and 24 hours continuous treatment and 8.0% in the short-term treatment with S9 mix.

2. CHROMOSOMAL ABERRATION TEST

2.1 Short-term Treatment (Tables 2, 3, 4 and Figs. 2, 3)

1) Without S9 mix

(1) Cell growth rate and IC_{50}

The cell growth rates at 26.6, 37.2, 52.1, 72.9, 102, 143, 200 and 280 μg/mL of the test substance were 106.2, 88.8, 81.4, 60.6, 39.3, 28.5, 29.3 and 26.1%, respectively. The IC_{50} was calculated with 87 μg/mL.

(2) Precipitation of the test substance, color of medium and corrosion of culture dish

Precipitation of the test substance was observed at 102 μg/mL or more at the start and the end of the treatment. Color change of the medium and the corrosion of the culture dish were not observed at all doses.

(3) Frequency of cells with structural aberrations

The frequencies of cells with structural aberrations were 1.5% in the negative control and 47.0% in the positive control. The frequencies of cells with structural aberrations at 52.1, 72.9 and 102 μg/mL were 0.5, 27.0 and 3.0%, respectively, though the frequencies was not recognized with a dose-related increase, the cell with structural aberrations was showed 10% or more.

(4) Frequency of numerical aberration cells

The frequencies of numerical aberration cells were below 5.0% at all doses including the negative and the positive controls, respectively, therefore, the results were judged to be negative.

2) With S9 mix

(1) Cell growth rate and IC_{50}

The cell growth rates at 26.6, 37.2, 52.1, 72.9, 102, 143, 200 and 280 μg/mL of the test substance were 96.5, 89.7, 92.1, 72.6, 56.3, 28.3, 13.5 and 5.2%, respectively. The IC_{50} was calculated with 110 μg/mL.

(2) Precipitation of the test substance, color of medium and corrosion of culture dish

Precipitation of the test substance was observed at 102 μg/mL or more at the start and the end of the treatment. Color change of the medium and the corrosion of the culture dish were not observed at all doses.

(3) Frequency of cells with structural aberrations

The frequencies of cells with structural aberrations were 2.5% in the negative control and 50.0% in the positive control. The frequencies of cells with structural aberrations at 72.9, 102 and 143 μg/mL were 3.0, 4.0 and 4.0%, respectively, therefore, the results were judged to be negative.

(4) Frequency of numerical aberration cells

The frequencies of numerical aberration cells were below 5.0% at all doses including the negative and the positive controls, respectively, therefore, the results were judged to be negative.

2.2 Twenty Four Hours Continuous Treatment (Tables 2, 5 and Figs. 2, 4)

1) Cell growth rate and IC_{50}

The cell growth rates at 19.0, 26.6, 37.2, 52.1, 72.9, 102, 143 and 200 μg/mL of the test substance were 95.7, 79.5, 69.2, 58.9, 41.8, 41.5, 19.8 and 14.4%, respectively. The IC_{50} was calculated with 63 μg/mL.

2) Precipitation of the test substance, color of medium and corrosion of culture dish

Precipitation of the test substance was observed at 102 μg/mL or more at the start and the end of the treatment. Color change of the medium and the corrosion of the culture dish were not observed at all doses.

3) Frequency of cells with structural aberrations

The frequencies were 1.5% in the negative control and 75.0% in the positive control. The frequencies of cells with structural aberrations at 37.2, 52.1, 72.9 and 102 μg/mL were 0.5, 3.0, 7.5 and 15.5%, respectively, therefore, cell with structural aberrations was showed 10% or more with a dose-related increase, therefore, the results were judged to be positive.

4) Frequency of numerical aberration cells

The frequencies of numerical aberration cells were below 5.0% at all doses including the negative and the positive controls, respectively, therefore, the

results were judged to be negative.

5) D_{20} value

It was calculated at 0.16 mg/mL with structural aberration.

3. CONFIRMATION TEST IN THE SHORT-TERM TREATMENT WITHOUT S9 MIX (TABLES 6, 7 AND FIGS. 5, 6)

3.1 Cell Growth Rate and IC_{50}

The cell growth rates at 41.9, 50.2, 60.3, 72.3, 86.8, 104, 125 and 150 μg/mL of the test substance were 88.3, 80.0, 80.2, 59.5, 43.2, 42.5, 27.6 and 27.4%, respectively. The IC_{50} was calculated with 81 μg/mL.

3.2 Precipitation of the Test Substance, Color of Medium and Corrosion of Culture Dish

Precipitation of the test substance was observed at 104 μg/mL or more at the start and the end of the treatment. Color change of the medium and the corrosion of the culture dish were not observed at all doses.

3.3 Frequency of Cells with Structural Aberrations

The frequencies were 0.5% in the negative control and 59.0% in the positive control. The frequencies of cells with structural aberrations at 60.3, 72.3 and 86.8 μg/mL were 2.5, 3.0 and 2.5%, respectively, therefore, the results were judged to be negative.

3.4 Frequency of Numerical Aberration Cells

The frequencies of numerical aberration cells were below 5.0% at all doses including the negative and the positive controls, respectively, therefore, the results were judged to be negative.

4. TYPICAL PHOTOS

The normal cell was shown in photo 1, and cell with structural aberration induced by the test substance was shown in photos 2 and 3.

DISCUSSION AND CONCLUSION

In each treatment method, the frequencies of cells with aberrations were below 5% in the negative controls, and the frequencies of cells with structural aberrations excluding gaps were 20% or more in the positive controls. In 24 hours continuous treatment, though the frequencies of cells with structural aberrations at 102 μg/mL were 6.0 and 25.0% and fluctuated between two culture dishes, the frequencies showed 5% or more, therefore, it was judged that it did not fluctuated markedly. Therefore, the present study was

appropriately performed.

As a result of chromosomal aberration test, the frequencies of numerical aberration cells were below 5% at all observation doses of the test substance in each treatment, therefore, numerical aberration was judged to be negative. The frequencies of cells with structural aberrations were below 5% at åll observation doses of the test substance in the short-term treatment with S9 mix, though the frequency of cells with structural aberration was over 10% and the frequencies were recognized as a dose-related increase in 24 hours continuous treatment. Therefore, structural aberration was judged to be positive.

In 24 hours continuous treatment, the frequencies of cells with structural aberrations fluctuated between two culture dishes at the dose that the frequencies of cells with structural aberration increased. The cell growth rate was about 30% for one dish that the frequency of cells with structural aberration was 10% or more, on the other hand, the cell growth rate was about 50% for another dish that the frequency of cells with structural aberration was about 5%. Therefore, it was considered that the fluctuation of the frequencies resulted from the fluctuation of the cell growth rate. The fluctuation of the cell growth rates was shown at the dose that precipitation of the test substance was observed in the medium, therefore, it was judged that the fluctuation of the frequencies was occurred by slightly deference of the dispersion of the test substance in medium.

In the short-term treatment without S9 mix, the frequency of cells with structural aberrations showed 27.0% at 72.9 μg/mL in the chromosomal aberration test. However, the frequencies of cells with structural aberrations at 60.3, 72.3 and 86.8 μg/mL were below 5% in the confirmation test. The frequency of cells with structural aberration in the chromosomal aberration test was not recognized with a dose-related increase, therefore, it was judged that the increase of the structural aberration was accidental and structural aberration was judged to be negative in the short-term treatment without S9 mix. Additionally, in the short-term treatments without and with S9 mix, the frequencies of cells with structural aberration were 5% or more in the cell growth inhibition test. The number of cells for observation was only 50 cells per dose in the cell growth inhibition test. Because the cell growth rate was less than 30% or the test substance had not been dispersed in medium at the doses that the frequency of cells with structural aberration showed 5% or more, it was judged that the frequencies increased under an inappropriate condition for evaluation of the chromosomal aberration.

Based on the above results, it was considered that 13F-SFA did not induce numerical aberration but induced structural aberration under the present test conditions.

REFERENCES

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2. Japan Environmental Mutagenicity Society/Mammalian Mutagenicity Study Group (ed.) (1988) Atlas of chromosomal aberration induced with chemical substance (in Japanese). Asakura Publishing Co., Tokyo.

Table 1 Results of cell growth inhibition test of 13F-SFA

Substance	Dose (μg/mL)	Treatment-recovery time (hour)	S9 mix	Cell growth rate (%)	Precipitation of test substance in medium [a]			Frequency of cells with aberrations (%)[b]	
					Treatment start	Treatment end	Culture end	Structural aberration	Numerical aberration
Dehydrated acetone	0	6-18	-	100	-	-	-	0.0	0.0
13F-SFA	16.3	6-18	-	100.5	-	-	-	n.o.	n.o.
	32.7	6-18	-	90.0	-	-	-	n.o.	n.o.
	65.3	6-18	-	61.6	-	-	-	0.0	0.0
	131	6-18	-	23.2	+	+	-	6.0	0.0
	261	6-18	-	28.2	+	+	-	10.0	0.0
	523	6-18	-	23.2	+	+	-	few meta	
	1050	6-18	-	31.9	+	+	+	4.0	0.0
	2090	6-18	-	38.8	+	+	+	4.0	0.0
	4180	6-18	-	36.9	+	+	+	2.0	0.0
				IC_{50} : 85 μg/mL					
Dehydrated acetone	0	6-18	+	100	-	-	-	0.0	0.0
13F-SFA	16.3	6-18	+	106.7	-	-	-	n.o.	n.o.
	32.7	6-18	+	106.4	-	-	-	n.o.	n.o.
	65.3	6-18	+	88.8	-	-	-	0.0	0.0
	131	6-18	+	21.9	+	+	-	6.0	0.0
	261	6-18	+	5.1	+	+	-	few meta	
	523	6-18	+	17.8	+	+	-	8.0	2.0
	1050	6-18	+	35.1	+	+	-	2.0	2.0
	2090	6-18	+	54.4	+	+	-	6.0	2.0
	4180	6-18	+	46.0	+	+	-	8.0	0.0
				IC_{50} : 100 μg/mL					
Dehydrated acetone	0	24-0	-	100	-	-		0.0	0.0
13F-SFA	16.3	24-0	-	84.2	-	-		n.o.	n.o.
	32.7	24-0	-	68.3	-	-		0.0	0.0
	65.3	24-0	-	46.3	-	-		4.0	2.0
	131	24-0	-	15.2	+	-		few meta	
	261	24-0	-	9.7	+	+		no meta	
	523	24-0	-	11.0	+	+		no meta	
	1050	24-0	-	11.1	+	+		no meta	
	2090	24-0	-	16.4	+	+		8.0	0.0
	4180	24-0	-	14.9	+	+		10.0	0.0
				IC_{50} : 60 μg/mL					

Acetone was dehydrated with molecular sieves 5A 1/8.

n.o.: not observed, few meta: the frequency of metaphases was extremely few., no meta: metaphases were not observed.

a) Precipitation of the test substance: -, absence; +, presence

b) The frequency of cells with chromosomal aberrations was calculated by observating 50 metaphases per dose.

note) The dose was set at 4180 μg/mL as equivalent to 10 mmol/L, as the maximum dose in the case of no cytotoxicity, the dose levels based on a geometric progression of 2 were selected.

Table 2 Results of chromosomal aberration test of 13F-SFA

Substance	Dose (μg/mL)	Treatment-recovery time (hour)	S9 mix	Cell growth rate (%)	Precipitation of test substance in medium [a]			Frequency of cells with aberrations (%)[b]	
					Treatment start	Treatment end	Culture end	Structural aberration	Numerical aberration
Dehydrated acetone	0	6-18	-	100	-	-	-	1.5	1.0
13F-SFA	26.6	6-18	-	106.2	-	-	-	n.o.	n.o.
	37.2	6-18	-	88.8	-	-	-	n.o.	n.o.
	52.1	6-18	-	81.4	-	-	-	0.5	0.0
	72.9	6-18	-	60.6	-	-	-	27.0	0.5
	102	6-18	-	39.3	+	+	-	3.0	0.0
	143	6-18	-	28.5	+	+	-	n.o.	n.o.
	200	6-18	-	29.3	+	+	-	n.o.	n.o.
	280	6-18	-	26.1	+	+	-	n.o.	n.o.
MMC	0.1	6-18	-	ND	-	-	-	47.0	1.5
				IC_{50} : 87 μg/mL					
Dehydrated acetone	0	6-18	+	100	-	-	-	2.5	1.0
13F-SFA	26.6	6-18	+	96.5	-	-	-	n.o.	n.o.
	37.2	6-18	+	89.7	-	-	-	n.o.	n.o.
	52.1	6-18	+	92.1	-	-	-	n.o.	n.o.
	72.9	6-18	+	72.6	-	-	-	3.0	1.5
	102	6-18	+	56.3	+	+	-	4.0	1.0
	143	6-18	+	28.3	+	+	-	4.0	1.5
	200	6-18	+	13.5	+	+	-	few meta	
	280	6-18	+	5.2	+	+	-	no meta	
CPA	6	6-18	+	ND	-	-	-	50.0	1.0
				IC_{50} : 110 μg/mL					
Dehydrated acetone	0	24-0	-	100	-	-		1.5	0.0
13F-SFA	19.0	24-0	-	95.7	-	-		n.o.	n.o.
	26.6	24-0	-	79.5	-	-		n.o.	n.o.
	37.2	24-0	-	69.2	-	-		0.5	0.0
	52.1	24-0	-	58.9	-	-		3.0	0.0
	72.9	24-0	-	41.8	-	-		7.5	1.5
	102	24-0	-	41.5	+	+		15.5	0.5
	143	24-0	-	19.8	+	+		few meta	
	200	24-0	-	14.4	+	+		no meta	
MMC	0.05	24-0	-	ND	-	-		75.0	1.0
				IC_{50} : 63 μg/mL			D_{20} :	0.16 mg/mL	-

Acetone was dehydrated with molecular sieves 5A 1/8.

MMC: mitomycin C, CPA: cyclophosphamide monohydrate

ND: not detected

n.o.: not observed, few meta: the frequency of metaphases was extremely few., no meta: metaphases were not observed.

a) Precipitation of the test substance: -, absence; +, presence

b) The frequency of cells with chromosomal aberrations was calculated by observating 200 metaphases per dose.

Table 3 Results of chromosomal aberration test (short term-treatment without S9 mix)

Name of test substance : 13F-SFA

K06-1189

Treatment time (h)	S9 mix	Dose (μg/mL)	Number of cells with structural chromosomal aberrations (frequency%)							Number of gaps (frequency%)	Cell growth rate (%)	Number of cells with numerical chromosomal aberrations (frequency%)			
			Number of cells observed	Chromatid break	Chromatid exchange	Chromosome break	Chromosome exchange	Others	Total number of cells with aberrations			Number of cells observed	Polyploids	Others	Total number of cells with aberrations
6 - 18	-	Negative control (Acetone)	100	0	1	0	0	0	1	0	100	100	1	0	1
			100	1	1	0	0	0	2	0		100	1	0	1
		0	200	1 (0.5)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.5)	0 (0.0)		200	2 (1.0)	0 (0.0)	2 (1.0)
6 - 18	-	26.6	0								103.6	0			
			0								108.7	0			
			0								(106.2)	0			
6 - 18	-	37.2	0								94.7	0			
			0								82.8	0			
			0								(88.8)	0			
6 - 18	-	52.1	100	0	1	0	0	0	1	0	79.4	100	0	0	0
			100	0	0	0	0	0	0	0	83.3	100	0	0	0
			200	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	(81.4)	200	0 (0.0)	0 (0.0)	0 (0.0)
6 - 18	-	72.9	100	17	14	0	0	0	24	1	64.6	100	0	0	0
			100	20	21	0	0	0	30	0	56.6	100	1	0	1
			200	37 (18.5)	35 (17.5)	0 (0.0)	0 (0.0)	0 (0.0)	54 (27.0)	1 (0.5)	(60.6)	200	1 (0.5)	0 (0.0)	1 (0.5)
6 - 18	-	102 †	100	3	0	0	0	0	3	1	45.6	100	0	0	0
			100	2	1	0	0	0	3	0	33.0	100	0	0	0
			200	5 (2.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	6 (3.0)	1 (0.5)	(39.3)	200	0 (0.0)	0 (0.0)	0 (0.0)
6 - 18	-	143 †	0								29.6	0			
			0								27.4	0			
			0								(28.5)	0			
6 - 18	-	200 †	0								32.8	0			
			0								25.7	0			
			0								(29.3)	0			
6 - 18	-	280 †	0								22.1	0			
			0								30.1	0			
			0								(26.1)	0			
6 - 18	-	Positive control (MMC)	100	21	45	0	0	0	51	0		100	3	0	3
			100	15	38	1	1	0	43	2		100	0	0	0
		0.1	200	36 (18.0)	83 (41.5)	1 (0.5)	1 (0.5)	0 (0.0)	94 (47.0)	2 (1.0)		200	3 (1.5)	0 (0.0)	3 (1.5)

Treatment time comprised treatment-time and recovery-time.

The number of aberrant cells at each dish was shown at the first and the second lines. The total number of them was shown at the third line.

Cell growth rate at each dish was shown at the first and the second lines. The average of them was shown at the third line.

Acetone was dehydrated with molecular sieves 5A 1/8.

MMC: Mitomycin C

†: Precipitation of the test substance was observed at the start and the end of the treatment.

The specimens at 26.6, 37.2, 143, 200 and 280 μg/mL were not observed.

Table 4 Results of chromosomal aberration test (short-term treatment with S9 mix)

Name of test substance : 13F-SFA

K06-1189

Treatment time (h)	S9 mix	Dose (μg/mL)	Number of cells with structural chromosomal aberrations (frequency%)							Number of gaps (frequency%)	Cell growth rate (%)	Number of cells with numerical chromosomal aberrations (frequency%)			
			Number of cells observed	Chromatid break	Chromatid exchange	Chromosome break	Chromosome exchange	Others	Total number of cells with aberrations			Number of cells observed	Polyploids	Others	Total number of cells with aberrations
6 - 18	+	Negative control (Acetone) 0	100	4	0	0	0	0	4	0	100	100	1	0	1
			100	1	0	0	0	0	1	0		100	1	0	1
			200	5 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (2.5)	0 (0.0)		200	2 (1.0)	0 (0.0)	2 (1.0)
6 - 18	+	26.6	0								97.1	0			
			0								95.8	0			
			0								(96.5)	0			
6 - 18	+	37.2	0								90.6	0			
			0								88.7	0			
			0								(89.7)	0			
6 - 18	+	52.1	0								94.1	0			
			0								90.0	0			
			0								(92.1)	0			
6 - 18	+	72.9	100	0	1	0	1	0	2	0	73.8	100	1	0	1
			100	3	1	0	0	0	4	0	71.3	100	2	0	2
			200	3 (1.5)	2 (1.0)	0 (0.0)	1 (0.5)	0 (0.0)	6 (3.0)	0 (0.0)	(72.6)	200	3 (1.5)	0 (0.0)	3 (1.5)
6 - 18	+	102 †	100	4	0	0	0	0	4	1	54.5	100	1	0	1
			100	3	1	0	0	0	4	2	58.1	100	1	0	1
			200	7 (3.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	8 (4.0)	3 (1.5)	(56.3)	200	2 (1.0)	0 (0.0)	2 (1.0)
6 - 18	+	143 †	100	2	0	0	0	0	2	1	28.4	100	1	0	1
			100	5	4	0	0	0	6	1	28.2	100	2	0	2
			200	7 (3.5)	4 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	8 (4.0)	2 (1.0)	(28.3)	200	3 (1.5)	0 (0.0)	3 (1.5)
6 - 18	+	200 †	0	few metaphases							12.8	0	few metaphases		
			0								14.2	0			
			0								(13.5)	0			
6 - 18	+	280 †	0	no metaphases							6.1	0	no metaphases		
			0								4.3	0			
			0								(5.2)	0			
6 - 18	+	Positive control (CPA) 6	100	16	38	0	0	0	50	1		100	1	0	1
			100	19	41	0	0	0	50	3		100	1	0	1
			200	35 (17.5)	79 (39.5)	0 (0.0)	0 (0.0)	0 (0.0)	100 (50.0)	4 (2.0)		200	2 (1.0)	0 (0.0)	2 (1.0)

Treatment time comprised treatment-time and recovery-time.

The number of aberrant cells at each dish was shown at the first and the second lines. The total number of them was shown at the third line.

Cell growth rate at each dish was shown at the first and the second lines. The average of them was shown at the third line.

Acetone was dehydrated with molecular sieves 5A 1/8.

CPA: Cyclophosphamide monohydrate

†: Precipitation of the test substance was observed at the start and the end of the treatment.

few metaphases: the frequency of metaphases was extremely few.

no metaphases: metaphases were not observed.

The specimens at 26.6, 37.2, 52.1, 200 and 280 μg/mL were not observed.

Table 5 Results of chromosomal aberration test (continuous treatment)

Name of test substance : 13F-SFA

K06-1189

Treatment time (h)	Dose (μg/mL)	Number of cells with structural chromosomal aberrations (frequency%)							Number of gaps (frequency%)	Cell growth rate (%)	Number of cells with numerical chromosomal aberrations (frequency%)			
		Number of cells observed	Chromatid break	Chromatid exchange	Chromosome break	Chromosome exchange	Others	Total number of cells with aberrations			Number of cells observed	Polyploids	Others	Total number of cells with aberrations
24 - 0	Negative control (Acetone)	100	1	0	1	0	0	2	0	100	100	0	0	0
		100	1	0	0	0	0	1	0		100	0	0	0
	0	200	2 (1.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	3 (1.5)	0 (0.0)		200	0 (0.0)	0 (0.0)	0 (0.0)
24 - 0	19.0	0								96.2	0			
		0								95.1	0			
		0								(95.7)	0			
24 - 0	26.6	0								80.6	0			
		0								78.3	0			
		0								(79.5)	0			
24 - 0	37.2	100	1	0	0	0	0	1	1	70.5	100	0	0	0
		100	0	0	0	0	0	0	0	67.9	100	0	0	0
		200	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.5)	(69.2)	200	0 (0.0)	0 (0.0)	0 (0.0)
24 - 0	52.1	100	0	0	0	0	0	0	0	57.4	100	0	0	0
		100	5	1	0	0	0	6	0	60.3	100	0	0	0
		200	5 (2.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	6 (3.0)	0 (0.0)	(58.9)	200	0 (0.0)	0 (0.0)	0 (0.0)
24 - 0	72.9	100	3	6	1	0	0	10	0	32.5	100	0	0	0
		100	3	1	1	0	0	5	0	51.1	100	3	0	3
		200	6 (3.0)	7 (3.5)	2 (1.0)	0 (0.0)	0 (0.0)	15 (7.5)	0 (0.0)	(41.8)	200	3 (1.5)	0 (0.0)	3 (1.5)
24 - 0	102 †	100	4	2	0	0	0	6	2	53.0	100	1	0	1
		100	20	6	0	0	0	25	1	30.0	100	0	0	0
		200	24 (12.0)	8 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	31 (15.5)	3 (1.5)	(41.5)	200	1 (0.5)	0 (0.0)	1 (0.5)
24 - 0	143 †	0								19.6	0			
		0								20.0	0			
		0								(19.8)	0			
24 - 0	200 †	0								13.7	0			
		0								15.0	0			
		0								(14.4)	0			
24 - 0	Positive control (MMC)	100	43	65	0	1	0	78	3		100	1	0	1
		100	42	57	0	0	0	72	1		100	1	0	1
	0.05	200	85 (42.5)	122 (61.0)	0 (0.0)	1 (0.5)	0 (0.0)	150 (75.0)	4 (2.0)		200	2 (1.0)	0 (0.0)	2 (1.0)

Treatment time comprised treatment-time and recovery-time.

The number of aberrant cells at each dish was shown at the first and the second lines. The total number of them was shown at the third line.

Cell growth rate at each dish was shown at the first and the second lines. The average of them was shown at the third line.

Acetone was dehydrated with molecular sieves 5A 1/8.

MMC: Mitomycin C

†: Precipitation of the test substance was observed at the start and the end of the treatment.

few metaphases: the frequency of metaphases was extremely few.

no metaphases: metaphases were not observed.

The specimens at 19.0, 26.6, 143 and 200 μg/mL were not observed.

Table 6 Results of confirmation test of 13F-SFA

Substance	Dose (μg/mL)	Treatment-recovery time (hour)	S9 mix	Cell growth rate (%)	Precipitation of test substance in medium [a]			Frequency of cells with aberrations (%)[b]	
					Treatment start	Treatment end	Culture end	Structural aberration	Numerical aberration
Dehydrated acetone	0	6-18	-	100	-	-	-	0.5	0.0
13F-SFA	41.9	6-18	-	88.3	-	-	-	n.o.	n.o.
	50.2	6-18	-	80.0	-	-	-	n.o.	n.o.
	60.3	6-18	-	80.2	-	-	-	2.5	0.0
	72.3	6-18	-	59.5	-	-	-	3.0	0.0
	86.8	6-18	-	43.2	-	-	-	2.5	0.5
	104	6-18	-	42.5	+	+	-	n.o.	n.o.
	125	6-18	-	27.6	+	+	-	n.o.	n.o.
	150	6-18	-	27.4	+	+	-	n.o.	n.o.
MMC	0.1	6-18	-	ND	-	-	-	59.0	0.5
IC_{50} : 81 μg/mL									

Acetone was dehydrated with molecular sieves 5A 1/8.

MMC: mitomycin C

ND: not detected, n.o.: not observed

a) Precipitation of the test substance: -, absence; +, presence

b) The frequency of cells with chromosomal aberrations was calculated by observating 200 metaphases per dose.

Table 7 Results of confirmation test (short-term treatment without S9 mix)

Name of test substance : 13F-SFA K06-1189

Treatment time (h)	S9 mix	Dose (μg/mL)	Number of cells with structural chromosomal aberrations (frequency%)							Number of gaps (frequency%)	Cell growth rate (%)	Number of cells with numerical chromosomal aberrations (frequency%)			
			Number of cells observed	Chromatid break	Chromatid exchange	Chromosome break	Chromosome exchange	Others	Total number of cells with aberrations			Number of cells observed	Polyploids	Others	Total number of cells with aberrations
6 - 18	-	Negative control (Acetone) 0	100	0	0	0	0	0	0	0	100	100	0	0	0
			100	1	0	0	0	0	1	0		100	0	0	0
			200	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)		200	0 (0.0)	0 (0.0)	0 (0.0)
6 - 18	-	41.9	0								90.3	0			
			0								86.2	0			
			0								(88.3)	0			
6 - 18	-	50.2	0								74.5	0			
			0								85.4	0			
			0								(80.0)	0			
6 - 18	-	60.3	100	0	2	0	0	0	2	0	80.9	100	0	0	0
			100	3	0	0	0	0	3	0	79.5	100	0	0	0
			200	3 (1.5)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (2.5)	0 (0.0)	(80.2)	200	0 (0.0)	0 (0.0)	0 (0.0)
6 - 18	-	72.3	100	2	0	0	0	0	2	0	56.1	100	0	0	0
			100	3	1	0	0	0	4	0	62.8	100	0	0	0
			200	5 (2.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	6 (3.0)	0 (0.0)	(59.5)	200	0 (0.0)	0 (0.0)	0 (0.0)
6 - 18	-	86.8	100	1	2	0	0	0	3	1	45.0	100	1	0	1
			100	1	0	0	1	0	2	0	41.3	100	0	0	0
			200	2 (1.0)	2 (1.0)	0 (0.0)	1 (0.5)	0 (0.0)	5 (2.5)	1 (0.5)	(43.2)	200	1 (0.5)	0 (0.0)	1 (0.5)
6 - 18	-	104 †	0								45.6	0			
			0								39.4	0			
			0								(42.5)	0			
6 - 18	-	125 †	0								26.5	0			
			0								28.7	0			
			0								(27.6)	0			
6 - 18	-	150 †	0								23.8	0			
			0								31.0	0			
			0								(27.4)	0			
6 - 18	-	Positive control (MMC) 0.1	100	26	56	0	0	0	64	0		100	1	0	1
			100	31	42	0	0	0	54	2		100	0	0	0
			200	57 (28.5)	98 (49.0)	0 (0.0)	0 (0.0)	0 (0.0)	118 (59.0)	2 (1.0)		200	1 (0.5)	0 (0.0)	1 (0.5)

Treatment time comprised treatment-time and recovery-time.

The number of aberrant cells at each dish was shown at the first and the second lines. The total number of them was shown at the third line.

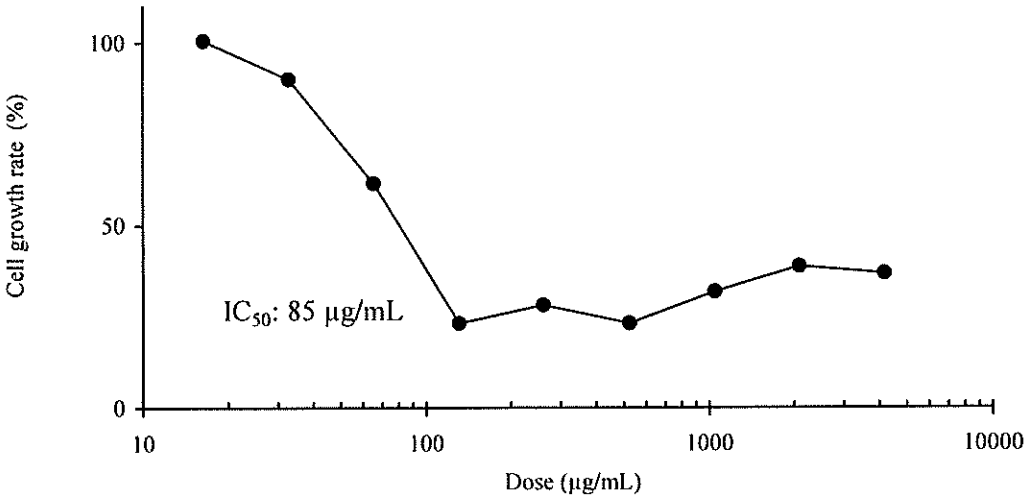
Cell growth rate at each dish was shown at the first and the second lines. The average of them was shown at the third line.

Acetone was dehydrated with molecular sieves 5A 1/8.

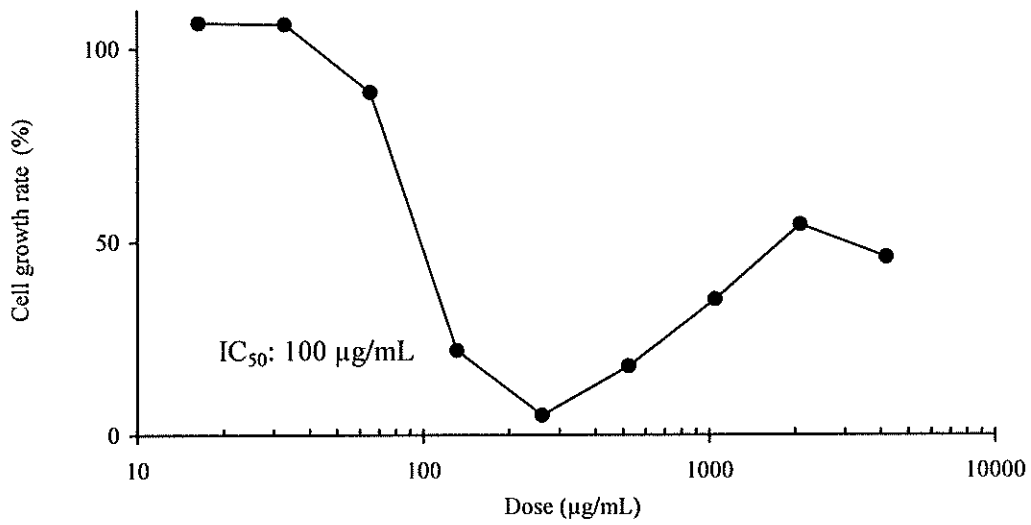
MMC: Mitomycin C

†: Precipitation of the test substance was observed at the start and the end of the treatment.

The specimens at 41.9, 50.2, 104, 125 and 150 μg/mL were not observed.



Short-term treatment without S9 mix



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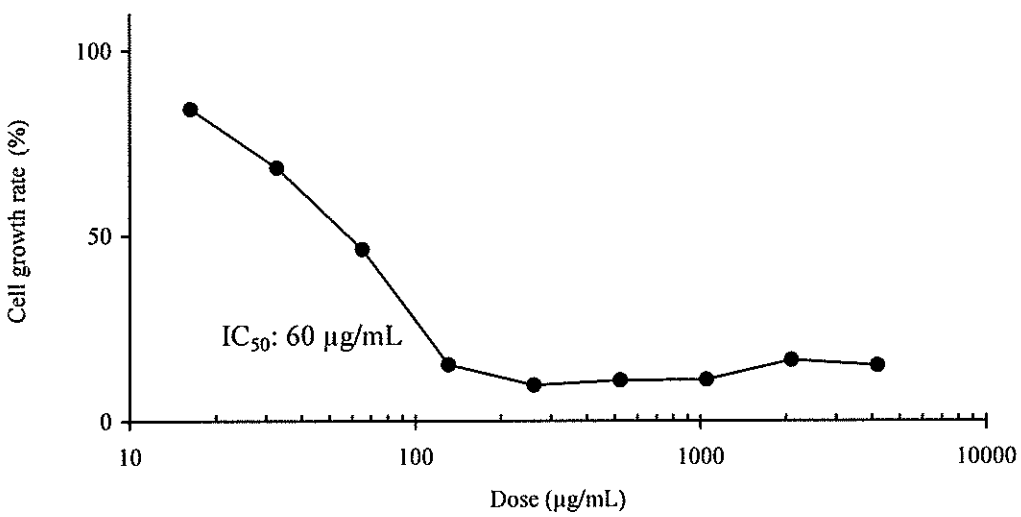
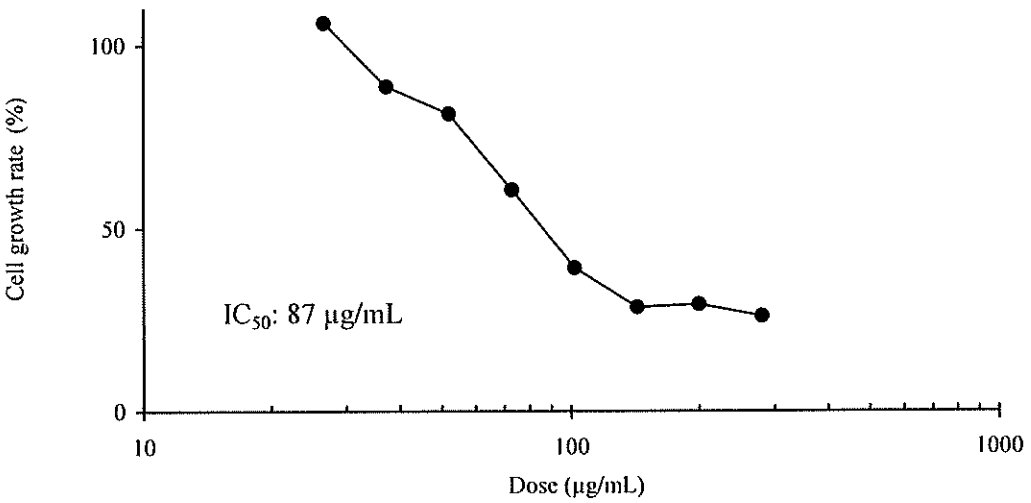
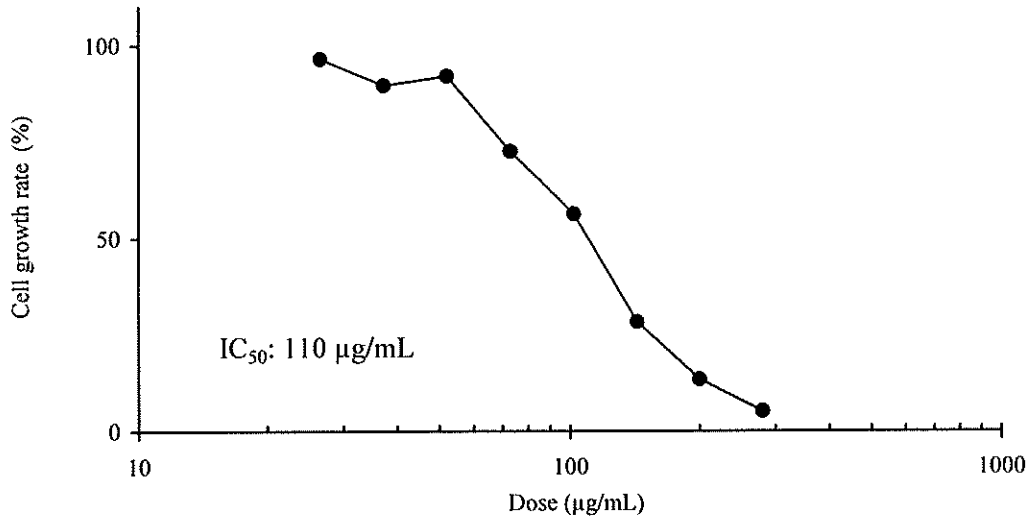


24 hours continuous treatment

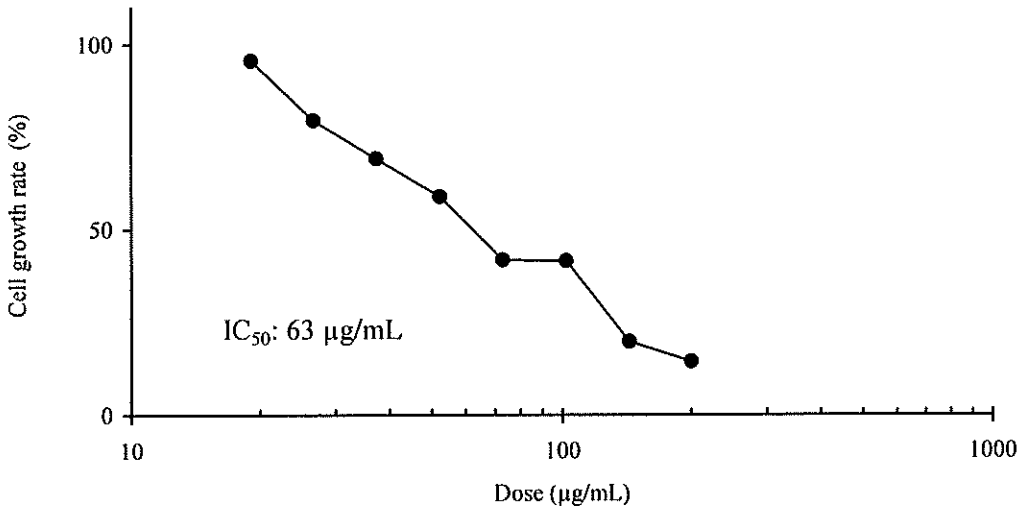
Fig. 1 Results of cell growth inhibition test of 13F-SFA



Short-term treatment without S9 mix

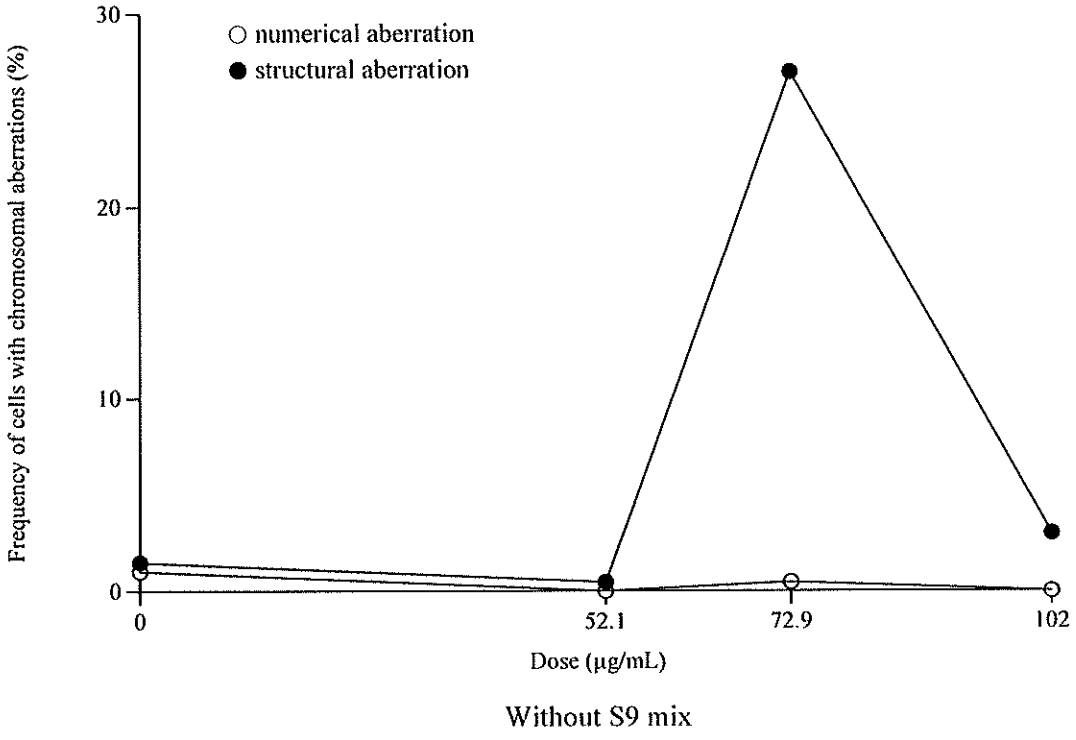


Short-term treatment with S9 mix

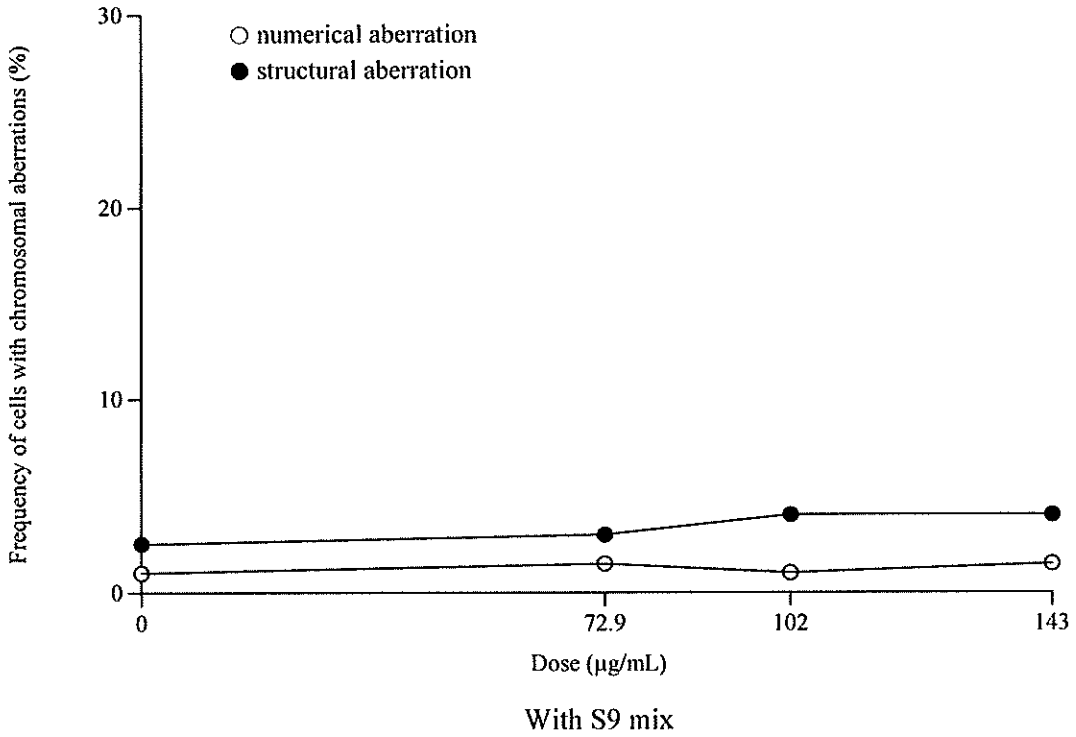


24 hours continuous treatment

Fig. 2 Cell growth rate in chromosomal aberration test of 13F-SFA



Without S9 mix



With S9 mix

Fig. 3 Results of chromosomal aberration test in short-term treatments of 13F-SFA

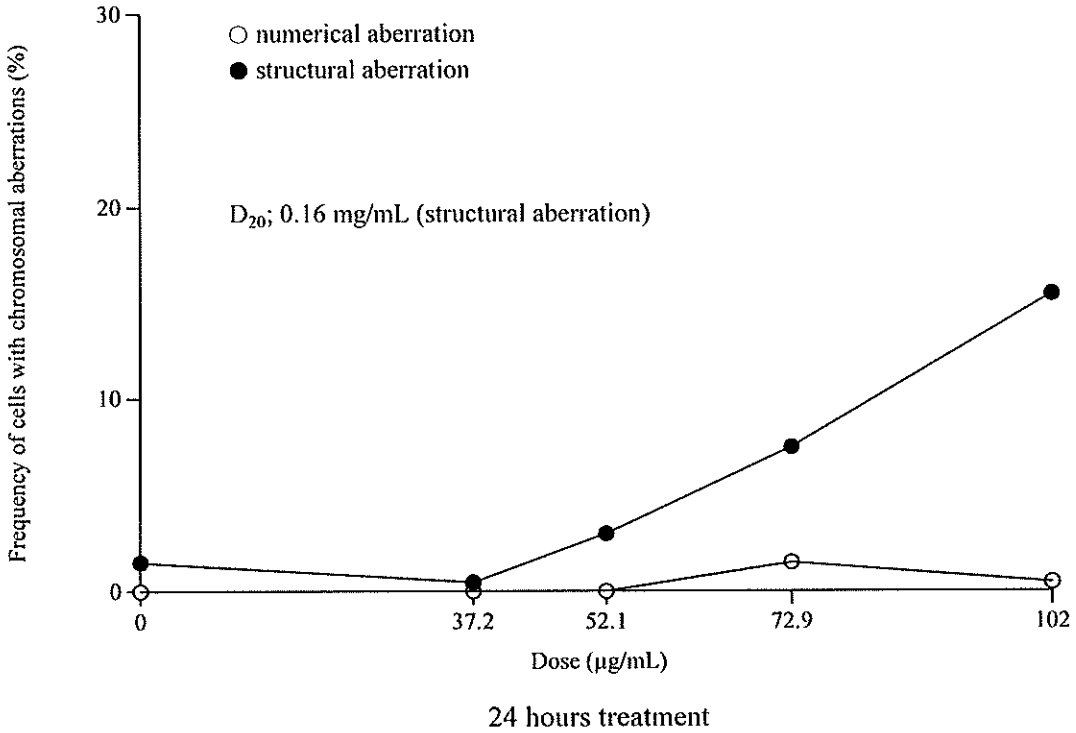
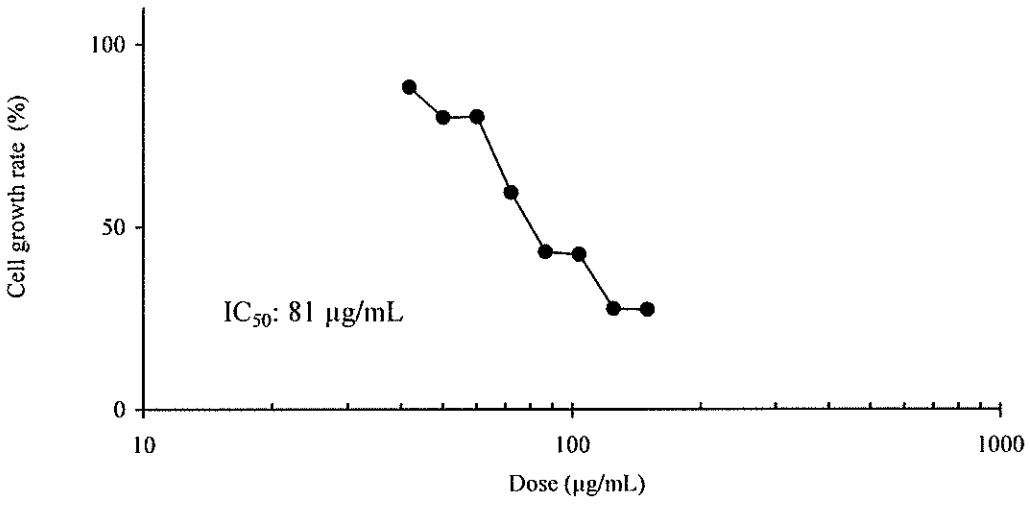


Fig. 4 Results of chromosomal aberration test in continuous treatment of 13F-SFA



Short-term treatment without S9 mix

Fig. 5 Cell growth rate in confirmation test of 13F-SFA

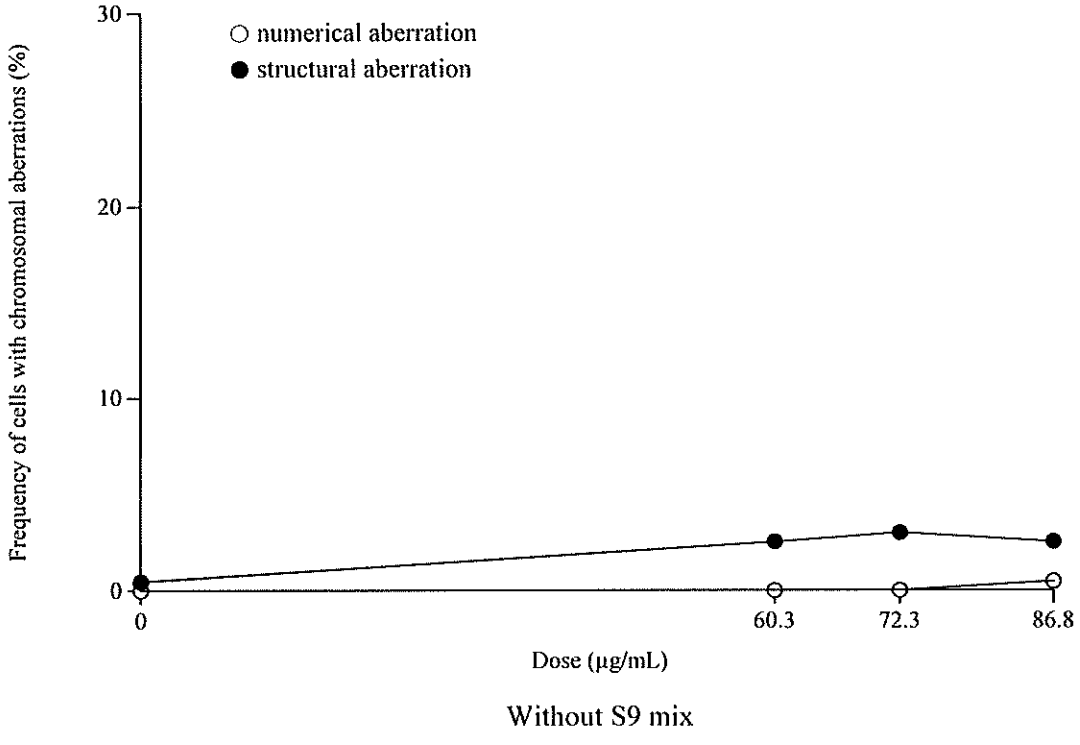


Fig. 6 Results of confirmation test in short-term treatment of 13F-SFA

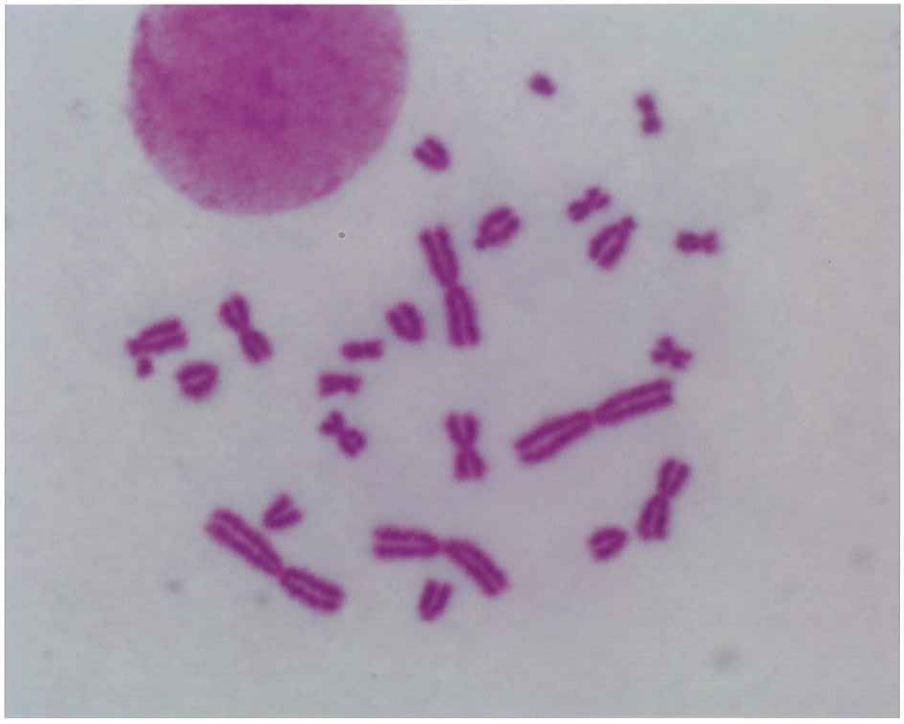


Photo 1 Normal cell
Negative control in 24 hours continuous treatment

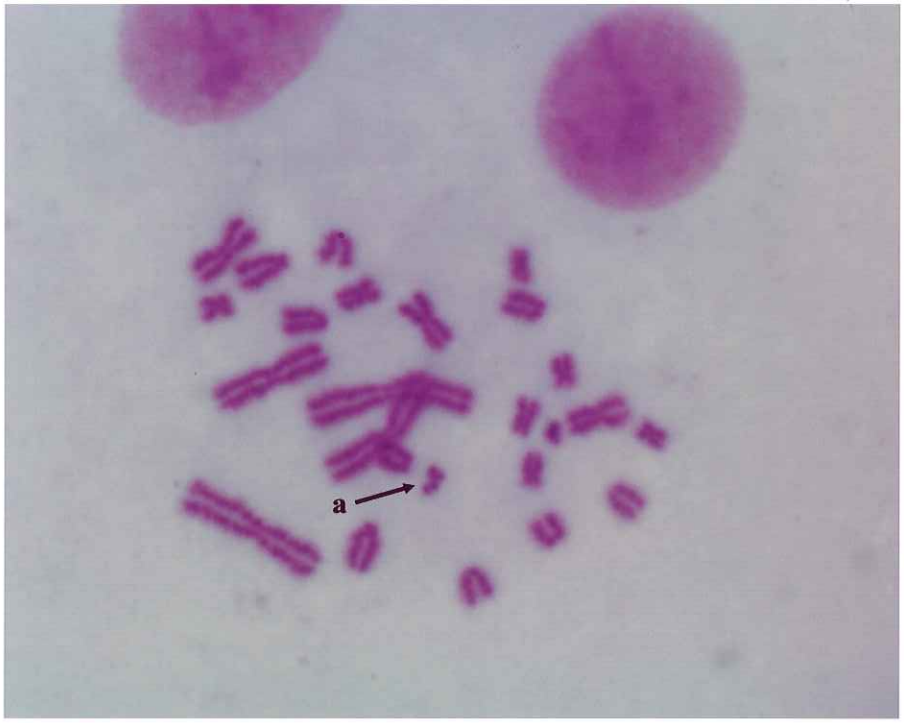


Photo 2 Structural aberration induced by 13F-SFA
102 μg/mL in 24 hours continuous treatment
a : chromatid break

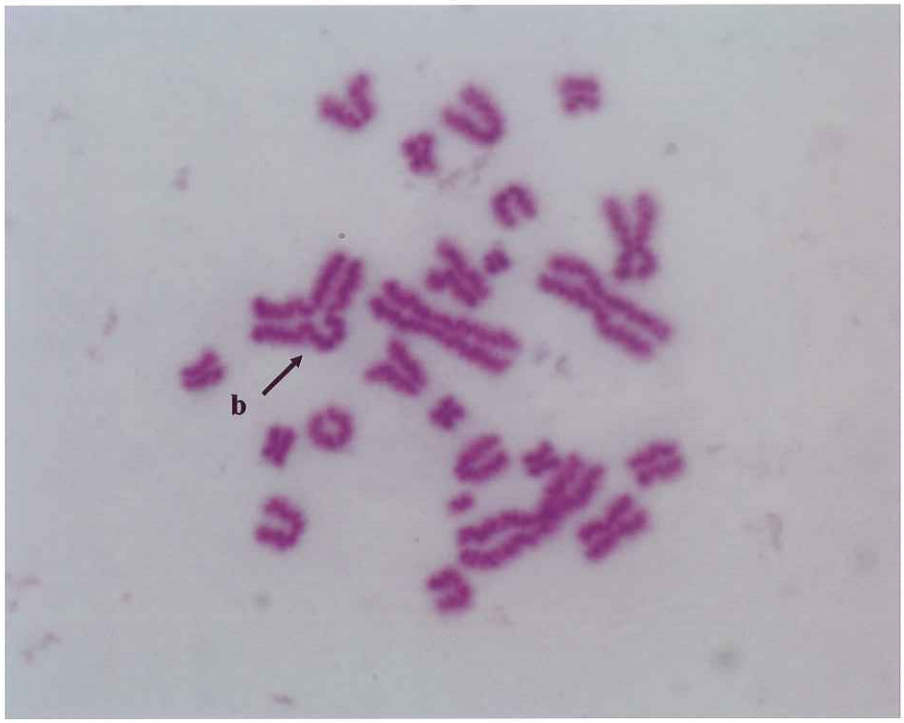


Photo 3 Structural aberration induced by 13F-SFA
72.9 μg/mL in 24 hours continuous treatment
b : chromatid exchange