

STATEMENT

TITLE OF STUDY

Chromosomal Aberration Test of 13F-OLE Using Cultured Mammalian Cells
(Study Code: K06-1191)

I, the undersigned, hereby declare that this report provides a correct English translation of the final report (Study Code: K06-1191, issued on March 14, 2007) audited by Quality Assurance Unit of CERI Hita, Chemicals Evaluation and Research Institute, Japan.

Makoto Nakai

Makoto Nakai
CERI Hita

October 1, 2009

Date

Chemicals Evaluation and Research Institute, Japan



Receipt No.837-06-T-5258

STUDY CODE: K06-1191

FINAL REPORT

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

March 2007

CERI Hita
Chemicals Evaluation and Research Institute, Japan

GLP STATEMENT

CERI Hita
Chemicals Evaluation and Research Institute, Japan

Sponsor: DAIKIN INDUSTRIES, LTD

Title: Chromosomal Aberration Test of 13F-OLE Using Cultured Mammalian
Cells

Study Code: K06-1191

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 14, 2007
Syozo Ajimi

QUALITY ASSURANCE STATEMENT

CERI Hita

Chemicals Evaluation and Research Institute, Japan

Sponsor: DAIKIN INDUSTRIES, LTD.Title: Chromosomal Aberration Test of 13F-OLE Using Cultured Mammalian CellsStudy Code: K06-1191

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

Phase	Date of Inspection	Date Reported to Study Director and Management
Protocol	December 12, 2006	December 12, 2006
Preparation of Test Substance	December 13, 2006	December 13, 2006
Treatment of Cells	December 13, 2006	December 13, 2006
Protocol Amendment	December 13, 2006	December 13, 2006
Reinspection of Protocol	December 15, 2006	December 16, 2006
Protocol Amendment (No. 2)	December 15, 2006	December 16, 2006
Protocol Amendment (No. 3)	December 20, 2006	December 20, 2006
Record of Accident or Variation from Protocol	December 20, 2006	December 20, 2006
Protocol Amendment (No. 4)	January 12, 2007	January 12, 2007
Protocol Amendment (No. 5)	January 21, 2007	January 22, 2007
Protocol Amendment (No. 6)	January 30, 2007	January 30, 2007
Raw Data and Draft Final Report	March 12, 2007	March 12, 2007
Reinspection of Raw Data and Draft Final Report	March 12, 2007	March 12, 2007
Draft Final Report (Second Time)	March 12, 2007	March 12, 2007
Reinspection of Draft Final Report (Second Time)	March 13, 2007	March 13, 2007
Final Report	March 14, 2007	March 14, 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.

Phase	Date of Inspection	Date Reported to Study Director and Management
Preparation and Management of Positive Control Substance	November 24, 2006	March 14, 2007
Preparation of Medium and Reagent	December 6 and 7, 2006	March 14, 2007
Cell Pre-culture	November 27, 2006	March 14, 2007
Collection of Cells and Preparation of Specimens	December 5, 2006	March 14, 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 14, 2007
Ryuichiro Mizuguchi

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Study Code: K06-1191

Test Substance Code: HR6853

Sponsor Code: D-0060

TITLE

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

SPONSOR

DAIKIN INDUSTRIES, LTD.

1-1, Nishi-Hitotsuya, Settsu, Osaka 566-8585, Japan

TESTING FACILITY

CERI Hita

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 8, 2006
Initiation of Experiment (Initiation of Cell Growth Inhibition Test):	December 13, 2006
Completion of Experiment (Completion of Observation of Specimens):	February 28, 2007
Completion of Study:	March 14, 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 the 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 will be recognized to be accurate copy will be sent to the sponsor.

STUDY DIRECTOR AND PERSONS CONCERNED WITH THE STUDY AND THE OPERATION

Study Director: Syozo Ajimi
Section 3, CERi Hita

Persons Concerned with the Study and Their Operation:

Junko Kawaguchi
(Preparation of test substance solution, cell treatment and
microscopic observation of specimens)
Syozo Ajimi
(Microscopic observation of specimens)

APPROVAL BY AUTHOR

Study Director: Signed in original March 14, 2007
Syozo Ajimi

SUMMARY

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

Based on the result of cell growth inhibition test, the doses in the chromosomal aberration test were set at 108, 216, 433, 865, 1730 and 3460 $\mu\text{g}/\text{mL}$ in the short-term treatments without and with S9 mix and the 24 hours continuous treatment.

In chromosomal aberration test, the observation doses for evaluation were selected at 865, 1730 and 3460 $\mu\text{g}/\text{mL}$ in all treatment methods. Then, the frequencies of cells with structural aberrations and numerical aberration cells were examined.

As a result of observation of specimens, the frequencies of cells with structural aberrations and numerical aberration cells were below 5% at all observation doses of the test substance in the short-term treatments without and with S9 mix and the 24 hours continuous treatment, therefore, both structural and numerical aberrations were judged to be negative.

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

It was concluded that 13F-OLE did not induce the chromosomal 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-Tridecafluoro-octa-1-ene

Other name: 13F-OLE

CAS No.: 25291-17-2

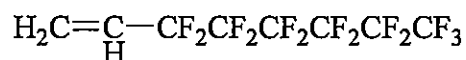
2) Lot No.

061122HM

3) Supplier

DAIKIN INDUSTRIES, LTD.

4) Structural formula



(Molecular formula: C₈H₃F₁₃)

5) Purity

99.8%

6) Names and concentrations of impurities

Unknown 0.2%

7) Physicochemical properties

Appearance at ordinary temperature:	Colorless transparent liquid
Molecular weight:	346.09
Stability:	—
Melting point:	—
Boiling point:	106°C at 760 mmHg
Vapor pressure:	—
Density	1.560 g/cm ³ at 20°C
Partition coefficient (1-octanol/water):	—
Hydrolyzability:	Unknown
Solubility	—
Degree of solubility	
Water:	Insoluble
Dimethyl sulfoxide (DMSO):	Insoluble
Acetone:	≥346 mg/mL
	(measured at the testing facility)
Others:	—

8) Storage conditions

Stored in a dark place at room temperature (cabinet No. 1 in the test substance storage room, permissible range: 10-30°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, permissible range: 10-30°C) and CPA in a cold dark place (refrigerator No.13 in the test substance storage room, permissible range: 1-10°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 spontaneous frequencies of cells with structural aberrations and the numerical aberration cells were below 5%.

CHL/IU cells have been recommended the availability on *in vitro* chromosome aberration test prescribed in "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% DMSO and were frozen in liquid nitrogen.

2.3 Culture Condition

Cells were cultured in a CO₂ incubator (MCO-345, SANYO Electric Co., Ltd. and Model 530, Wakenyaku Co., Ltd.), which was set at 37°C and 5% CO₂ 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 7 for the cell growth inhibition test, 9 for the first, 16 for the second and 18 for the third chromosomal aberration 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.) and basal medium (MEM) was prepared. 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, S9 protein content: 20.2 mg/mL, Oriental Yeast Co., Ltd.), which was prepared from livers of 7-week-old male SD rats (body weight: 215.1±10.7 g) administered intraperitoneally 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 MgCl₂, 33 µmol KCl, 5 µmol G-6-P, 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 Petri dish (Asahi Techno Glass Corporation) was used for cell culture.

Five milliliter of a cell suspension of 1.5×10^4 cells/mL were seeded into a dish and were cultured continuously for 2 days in the cell growth inhibition test and the first chromosomal aberration test. Five milliliter of a cell suspension of 5.0×10^3 cells/mL

were seeded into a dish and were cultured continuously for 3 days in the second and the third chromosomal aberration tests.

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.)

2) Reason for selection of solvent

The test substance was not soluble in water and DMSO as preinformed by the sponsor. The test substance was soluble in acetone at 346 mg/mL and was not indicated any change in color nor exothermic at room temperature within 2 hours after preparation. Therefore, acetone was selected as a solvent in this study.

3) Preparation method

After the test substance was weighed, acetone was added to the test substance to make an original solution using a laboratory mixer. The test substance solutions of 100 times concentrations of the test substance in the medium were prepared with the solvent. The test substance solutions were prepared under the yellow light. It was prepared without correcting purity of the test substance because the purity of the test substance was above 95%.

4) Preparation time

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

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 hour. 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 the 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 $\mu\text{g}/\text{mL}$ 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 2 mL of 0.25 w/v% trypsin. 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 collected by a centrifugation at 1000 rpm ($185\times g$) 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 glass slide, 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 3460 $\mu\text{g}/\text{mL}$ equivalent to 10 mmol/L, as the maximum dose in the case of no cytotoxicity on the guideline, and 13.5, 27.0, 54.1, 108, 216, 433, 865, 1730 and 3460 $\mu\text{g}/\text{mL}$ were set based on a

geometric progression of 2, respectively. Duplicate dishes were used for each dose.

3) Observation and scoring

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

(1) Structural aberration

The number of cells 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 cells 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 (two 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 hours 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 of the test substance

As the results of the cell growth inhibition test, in the short-term treatments without and with S9 mix and the 24 hours continuous treatment, because a cytotoxicity that the cell growth rate was below 50% was not obtained, the highest dose was selected at 3460 µg/mL equivalent to 10 mmol/L and the following 6 doses were set based on a geometric progression of 2.

Treatment method		Setting doses of test substance
Short-term treatment	Without S9 mix	108, 216, 433, 865, 1730 and 3460 µg/mL
	With S9 mix	108, 216, 433, 865, 1730 and 3460 µg/mL
24 hours continuous treatment		108, 216, 433, 865, 1730 and 3460 µg/mL

As the result of the chromosomal aberration test in the 24 hours continuous treatment employed above doses, as the cell growth inhibition test, it was confirmed that there were not sufficient number of metaphase cells necessary for observation at 1730 and 3460 µg/mL of test substance that the cell growth rate was approximately

80% or more. However, the test substance had water-shedding property and it was suspected that the cytotoxicity was caused by direct contact of the test substance with cells when collecting cells by centrifugation. Therefore, the second chromosomal aberration test was carried out in the 24 hours continuous treatment in which medium was exchanged before the addition of demecolcin solution two hours prior to the end of culture as the short-term treatments to remove the test substance. In the second test, same six doses were set as the first test.

As the result of the second chromosomal aberration test, there were not sufficient number of mitotic cells necessary for observation specimens at 1730 and 3460 $\mu\text{g/mL}$ and a fluctuation in the index of metaphase cells was observed between dishes at 433 and 865 $\mu\text{g/mL}$. It was considered that the fluctuation was caused by insufficient removal of the test substance when exchanging medium. Therefore, the third test was carried out under same procedure with the second test and the same six doses were set as the first and the second test.

Duplicate dishes were used for each dose.

3) Observation

(1) Dose for observation

Specimens for observation were selected from the first test in the short-term treatments without and with S9 mix and from the third test in the 24 hours continuous treatment in which sufficient metaphase cells for observation were appeared in all doses. All specimens of the negative and the positive controls set as the control groups in above tests were observed. The observation doses of the test substance were selected the consecutive doses of three stages. The highest observation dose in each treatment and the reason for selection are shown below.

In the short-term treatments without and with S9 mix and the 24 hours continuous treatment, cytotoxicity that the cell growth rate was less than 50% was not observed in any dose and it was able to observe chromosomal aberration to 3460 $\mu\text{g/mL}$ set as the highest dose. Therefore, 865, 1730 and 3460 $\mu\text{g/mL}$ were selected as the doses for observation of specimens in all treatment methods.

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

(2) Structural Aberration

Two hundred metaphase cells per dose (50 cells per specimen) containing 25 ± 2 chromosomes were observed 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 number of polyploid (cell with 38 or more chromosomes) cells among 200 metaphase cells per dose (50 cells per specimen) observed was recorded.

6.3 Confirmation Test

In the chromosomal aberration test for the short-term treatments without and with S9 mix and the 24 hours continuous treatment, the frequencies of cells with structural aberrations and numerical aberration cells were below 5% and the result was judged to be negative, therefore, a confirmation test was not conducted.

7. JUDGEMENT CRITERIA OF RESULTS

The findings were judged to be positive when the frequencies of cells with structural aberrations or numerical aberrations were 10% or more with a dose-related increase, or the frequencies of aberrant cells were 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.

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 of aberrant cells in the negative control were below 5%, and 3) the frequencies of cells with structural aberrations in the positive controls were 20% or more.

FACTORS AFFECTED RELIABILITY OF TEST

When weighing the test substance for the cell growth inhibition test, approximately 7.2 g of the test substance was spilled from the container. Spilled test substance was discarded and not used for the experiment. Furthermore, remaining amount of test substance was sufficient for all subsequent experiments. Therefore, it was considered that this accident did not affect the reliability of the test. There were no other factors, which might affect the reliability of the test.

TEST RESULTS

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

The IC₅₀s were calculated more than 3460 µg/mL in the short-term treatments without and with S9 mix and the 24 hours continuous treatment.

At the start and the end of the treatment, precipitation of the test substance was observed at 216 µg/mL or more in the short-term treatment without S9 mix and the 24 hours

continuous treatment and at 433 $\mu\text{g/mL}$ or more in the short-term treatment with S9 mix. At the end of the culture, it was observed at 433 $\mu\text{g/mL}$ or more in the short-term treatments without and with S9 mix. The color change of the medium and the corrosion of the culture dish were not observed at any doses.

The frequencies of cells with structural aberrations and numerical aberration cells were below 5% at all observation doses of the test substance in each treatment.

2. CHROMOSOMAL ABERRATION TEST

2.1 Short-term Treatment (Tables 2, 5, 6 and Figs. 2, 5)

1) Without S9 mix

(1) Cell growth rate and IC_{50}

The cell growth rates at 108, 216, 433, 865, 1730 and 3460 $\mu\text{g/mL}$ of the test substance were 92.2, 95.5, 95.6, 89.7, 94.0 and 90.4%, respectively. The IC_{50} was calculated more than 3460 $\mu\text{g/mL}$.

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

Precipitation of the test substance was observed at 216 $\mu\text{g/mL}$ or more at the start and the end of the treatment and the end of the culture. The color change of the medium and the corrosion of the culture dish were not observed at any doses.

(3) Frequency of cells with structural aberrations

The frequencies of cells with structural aberrations were 0.5% in the negative control and 64.5% in the positive control.

The frequencies of cells with structural aberrations at 865, 1730 and 3460 $\mu\text{g/mL}$ of the test substance were 3.5, 0.0 and 1.5%, respectively, therefore, the results were judged to be negative.

(4) Frequency of numerical aberration cells

The frequencies of numerical aberration cells were below 5% at all doses including the negative and positive controls, therefore, the results were judged to be negative.

2) With S9 mix

(1) Cell growth rate and IC_{50}

The cell growth rates at 108, 216, 433, 865, 1730 and 3460 $\mu\text{g/mL}$ of the test substance were 91.5, 81.5, 84.8, 74.9, 90.1 and 85.3%, respectively. The IC_{50} was calculated more than 3460 $\mu\text{g/mL}$.

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

Precipitation of the test substance was observed at 216 $\mu\text{g/mL}$ or more at the start and the end of the treatment and the end of the culture. The color change of the medium and the corrosion of the culture dish were not observed at any doses.

(3) Frequency of cells with structural aberrations

The frequencies of cells with structural aberrations were 0.0% in the negative control and 41.0% in the positive control.

The frequencies of cells with structural aberrations at 865, 1730 and 3460 $\mu\text{g/mL}$ of the test substance were 2.0, 1.5 and 1.0%, respectively. Therefore, the results were judged to be negative.

(4) Frequency of numerical aberration cells

The frequencies of numerical aberration cells were below 5% at all doses including the negative and positive controls, therefore, the results were judged to be negative.

2.2 Twenty Four Hours Continuous Treatment

1) The first test (Table 2 and Fig. 2)

(1) Cell growth rate and IC_{50}

The cell growth rates at 108, 216, 433, 865, 1730 and 3460 $\mu\text{g/mL}$ of the test substance were 92.2, 81.5, 81.6, 88.2, 94.6 and 79.6%, respectively. The IC_{50} was calculated more than 3460 $\mu\text{g/mL}$.

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

Precipitation of the test substance was observed at 216 $\mu\text{g/mL}$ or more at the start and the end of the treatment. The color change of the medium and the corrosion of the culture dish were not observed at any doses.

2) The second test (Table 3 and Fig. 3)

(1) Cell growth rate and IC_{50}

The cell growth rates at 108, 216, 433, 865, 1730 and 3460 $\mu\text{g/mL}$ of the test substance were 105.6, 108.9, 97.4, 95.6, 109.0 and 106.6%, respectively. The IC_{50} was calculated more than 3460 $\mu\text{g/mL}$.

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

Precipitation of the test substance was observed at 216 $\mu\text{g/mL}$ or more at the start and the end of the treatment and the end of the culture. The color change of the medium and the corrosion of the culture dish were not observed at any doses.

3) The third test (Tables 4, 7 and Figs. 4, 6)

(1) Cell growth rate and IC_{50}

The cell growth rates at 108, 216, 433, 865, 1730 and 3460 $\mu\text{g/mL}$ of the test substance were 95.2, 90.4, 87.8, 85.9, 87.5 and 91.0%, respectively. The IC_{50} was calculated more than 3460 $\mu\text{g/mL}$.

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

Precipitation of the test substance was observed at 216 $\mu\text{g/mL}$ or more at the start

and the end of the treatment and the end of the culture. The color change of the medium and the corrosion of the culture dish were not observed at any doses.

(3) Frequency of cells with structural aberrations

The frequencies of cells with structural aberrations were 2.0% in the negative control and 76.0% in the positive control.

The frequencies of cells with structural aberrations at 865, 1730 and 3460 $\mu\text{g/mL}$ were 1.0, 2.5 and 2.5%, respectively. Therefore, the results were judged to be negative.

(4) Frequency of numerical aberration cells

The frequencies of numerical aberration cells were below 5% at all doses including the negative and positive controls, therefore, the results were judged to be negative.

DISCUSSION AND CONCLUSION

In each treatment method in the chromosomal aberration test, the frequencies of cells with chromosomal aberrations did not fluctuate markedly between two culture dishes, and 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, indicating that the present study was appropriately performed.

As a result of observation of specimens, the frequencies of cells with structural aberrations and numerical aberration cells were below 5% at all observation doses of the test substance in the short-term treatments without and with S9 mix and the 24 hours continuous treatment, therefore, structural aberration and numerical aberration were judged to be negative.

Although the cell growth rates at 1730 and 3460 $\mu\text{g/mL}$ were approximately 80% or more in the 24 hours continuous treatment conducted with a regular procedure, there were not sufficient numbers of metaphase cells necessary for observation. However, it was able to obtain sufficient number of cells at all doses up to 3460 $\mu\text{g/mL}$ by exchanging medium two hours before the end of culture. Therefore, it was thought that the suppression of the indexes of metaphase cells was caused by the toxicity accompanied with direct contact of the test substance with cells when collecting cells by centrifugation. The ability to induce chromosomal aberration was evaluated under a test condition in which treatment medium was exchanged.

Based on the above results, it was considered that 13F-OLE did not induce the chromosomal aberration under the present test conditions.

REFERENCES

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

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
acetone	0	6-18	-	100	-	-	-	0.0	0.0
13F-OLE	13.5	6-18	-	98.8	-	-	-	n.o.	n.o.
	27.0	6-18	-	95.6	-	-	-	n.o.	n.o.
	54.1	6-18	-	91.4	-	-	-	n.o.	n.o.
	108	6-18	-	88.1	-	-	-	n.o.	n.o.
	216	6-18	-	78.9	+	+	-	0.0	0.0
	433	6-18	-	75.4	+	+	+	2.0	2.0
	865	6-18	-	68.0	+	+	+	0.0	0.0
	1730	6-18	-	75.6	+	+	+	4.0	2.0
	3460	6-18	-	84.1	+	+	+	2.0	0.0
	IC ₅₀ : >3460 µg/mL								
acetone	0	6-18	+	100	-	-	-	0.0	0.0
13F-OLE	13.5	6-18	+	91.1	-	-	-	n.o.	n.o.
	27.0	6-18	+	89.7	-	-	-	n.o.	n.o.
	54.1	6-18	+	99.6	-	-	-	n.o.	n.o.
	108	6-18	+	93.5	-	-	-	n.o.	n.o.
	216	6-18	+	88.9	-	-	-	n.o.	n.o.
	433	6-18	+	80.7	+	+	+	0.0	0.0
	865	6-18	+	84.0	+	+	+	4.0	2.0
	1730	6-18	+	87.5	+	+	+	0.0	2.0
	3460	6-18	+	74.7	+	+	+	0.0	0.0
	IC ₅₀ : >3460 µg/mL								
acetone	0	24-0	-	100	-	-		0.0	0.0
13F-OLE	13.5	24-0	-	95.1	-	-		n.o.	n.o.
	27.0	24-0	-	92.0	-	-		n.o.	n.o.
	54.1	24-0	-	92.1	-	-		n.o.	n.o.
	108	24-0	-	84.9	-	-		0.0	0.0
	216	24-0	-	74.2	+	+		0.0	0.0
	433	24-0	-	82.1	+	+		0.0	0.0
	865	24-0	-	84.0	+	+		few meta	
	1730	24-0	-	71.0	+	+		few meta	
	3460	24-0	-	78.6	+	+		few meta	
	IC ₅₀ : >3460 µg/mL								

n.o.: not observed, few meta: the frequency of metaphases was extremely few

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

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

The highest dose was set at 3460 µg/mL equivalent to 10 mmol/L, as the maximum dose in case of no cytotoxicity on the guidelines, the dose levels based on a geometric progression of 2 were selected.

Table 2 Results of the first chromosomal aberration test of 13F-OLE

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
acetone	0	6-18	-	100	-	-	-	0.5	0.0
13F-OLE	108	6-18	-	92.2	-	-	-	n.o.	n.o.
	216	6-18	-	95.5	+	+	+	n.o.	n.o.
	433	6-18	-	95.6	+	+	+	n.o.	n.o.
	865	6-18	-	89.7	+	+	+	3.5	2.5
	1730	6-18	-	94.0	+	+	+	0.0	0.5
	3460	6-18	-	90.4	+	+	+	1.5	1.0
MMC	0.1	6-18	-	ND	-	-	-	64.5	0.0
IC ₅₀ : >3460 µg/mL									
acetone	0	6-18	+	100	-	-	-	0.0	0.5
13F-OLE	108	6-18	+	91.5	-	-	-	n.o.	n.o.
	216	6-18	+	81.5	+	+	+	n.o.	n.o.
	433	6-18	+	84.8	+	+	+	n.o.	n.o.
	865	6-18	+	74.9	+	+	+	2.0	1.0
	1730	6-18	+	90.1	+	+	+	1.5	0.5
	3460	6-18	+	85.3	+	+	+	1.0	0.5
CPA	6	6-18	+	ND	-	-	-	41.0	0.5
IC ₅₀ : >3460 µg/mL									
acetone	0	24-0	-	100	-	-	-	n.o.	n.o.
13F-OLE	108	24-0	-	92.2	-	-	-	n.o.	n.o.
	216	24-0	-	81.5	+	+	+	n.o.	n.o.
	433	24-0	-	81.6	+	+	+	n.o.	n.o.
	865	24-0	-	88.2	+	+	+	n.o.	n.o.
	1730	24-0	-	94.6	+	+	+	few meta	
	3460	24-0	-	79.6	+	+	+	few meta	
MMC	0.05	24-0	-	ND	-	-	-	n.o.	n.o.
IC ₅₀ : >3460 µg/mL									

MMC: mitomycin C, CPA: cyclophosphamide monohydrate

ND: not detected, n.o.: not observed, few meta: the frequency of metaphases was extremely few

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

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

Table 3 Results of the second chromosomal aberration test of 13F-OLE

Substance	Dose ($\mu\text{g/mL}$)	Treatment- recovery time (hour)	S9 mix	Cell growth rate (%)	Precipitation of test substance in medium ^{a)}			Frequency of cells with aberrations (%)	
					Treatment start	Treatment end	Culture end	Structural aberration	Numerical aberration
acetone	0	24-0 ^{b)}	-	100	-	-	-	n.o.	n.o.
13F-OLE	108	24-0 ^{b)}	-	105.6	-	-	-	n.o.	n.o.
	216	24-0 ^{b)}	-	108.9	+	+	+	n.o.	n.o.
	433	24-0 ^{b)}	-	97.4	+	+	+	few meta ^{c)}	
	865	24-0 ^{b)}	-	95.6	+	+	+	few meta ^{c)}	
	1730	24-0 ^{b)}	-	109.0	+	+	+	few meta	
	3460	24-0 ^{b)}	-	106.6	+	+	+	few meta	
MMC	0.05	24-0 ^{b)}	-	ND	-	-	-	n.o.	n.o.
IC ₅₀ : >3460 $\mu\text{g/mL}$									

MMC: mitomycin C

ND: not detected

n.o.: not observed, few meta: the frequency of metaphases was extremely few

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

b) The medium was exchanged before the demecolcine solution was added 2 hours prior to cell harvest to remove the precipitation of the test substance.

c) It was confirmed that there were sufficient number of metaphase cells necessary for observation in the one culture dish, but it were not in the other.

Table 4 Results of the third chromosomal aberration test of 13F-OLE

Substance	Dose ($\mu\text{g/mL}$)	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
acetone	0	24-0 ^{c)}	-	100	-	-	-	2.0	3.0
13F-OLE	108	24-0 ^{c)}	-	95.2	-	-	-	n.o.	n.o.
	216	24-0 ^{c)}	-	90.4	+	+	+	n.o.	n.o.
	433	24-0 ^{c)}	-	87.8	+	+	+	n.o.	n.o.
	865	24-0 ^{c)}	-	85.9	+	+	+	1.0	0.5
	1730	24-0 ^{c)}	-	87.5	+	+	+	2.5	1.0
	3460	24-0 ^{c)}	-	91.0	+	+	+	2.5	2.0
MMC	0.05	24-0 ^{c)}	-	ND	-	-	-	76.0	0.5
IC ₅₀ : >3460 $\mu\text{g/mL}$									

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 observing 200 metaphases per dose.

c) The medium was exchanged before the demecolcine solution was added 2 hours prior to cell harvest to remove the precipitation of the test substance.

Table 5 Results of chromosomal aberration test (short-term treatment without S9 mix)

Treatment time (h)	S9 mix	Dose (µg/mL)	Number of cells with structural chromosomal aberrations (frequency%)							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 gaps (frequency%)	Cell growth rate (%)	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	0	0	0	
			100	0	1	0	0	0	1	0	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)	95.8 (92.2)	0	0 (0.0)	0 (0.0)	0 (0.0)
6 - 18	-	108	0	0	0	0	0	0	0	95.5 (92.2)	0	0	0	0	
			0	0	0	0	0	0	0	0	88.6 (92.2)	0	0	0	0
			0	0	0	0	0	0	0	0	95.5 (95.5)	0	0	0	0
6 - 18	-	216 †	0	0	0	0	0	0	0	93.6 (95.5)	0	0	0	0	
			0	0	0	0	0	0	0	0	97.5 (95.6)	0	0	0	0
			0	0	0	0	0	0	0	0	98.4 (98.4)	100	1	0	1
6 - 18	-	865 †	100	2	1	0	0	0	3	80.9 (89.7)	100	4	0	4	
			200	5 (2.5)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (3.5)	0 (0.0)	89.7 (89.7)	200	5 (2.5)	0 (0.0)	5 (2.5)
			100	0	0	0	0	0	0	0	98.0 (98.0)	100	0	0	0
6 - 18	-	1730 †	100	0	0	0	0	0	0	90.0 (94.0)	100	1	0	1	
			200	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	94.0 (94.0)	200	1 (0.5)	0 (0.0)	1 (0.5)
			100	0	0	0	0	0	0	0	91.9 (90.4)	100	1	0	1
6 - 18	-	3460 †	100	3 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.5)	88.8 (90.4)	100	1	0	1	
			200	36	55	0	0	0	0	3 (1.5)	90.4 (90.4)	200	2 (1.0)	0 (0.0)	2 (1.0)
			100	25	51	0	0	0	0	70	90.4 (90.4)	100	0	0	0
6 - 18	-	Positive control (MMC) 0.1	200	61 (30.5)	106 (53.0)	0 (0.0)	0 (0.0)	0 (0.0)	129 (64.5)	3 (1.5)	200	0 (0.0)	0 (0.0)	0 (0.0)	
			100	25	51	0	0	0	0	59	3	100	0	0	0
			200	61 (30.5)	106 (53.0)	0 (0.0)	0 (0.0)	0 (0.0)	129 (64.5)	3 (1.5)	200	0 (0.0)	0 (0.0)	0 (0.0)	

Treatment time comprised treatment-time and recovery-time.
 The number of aberrant cells at each dish was shown at the first and 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 second lines. The average of them was shown at the third line.
 MMC: Mitomycin C
 †: Precipitation of the test substance was observed at the start and the end of the treatment and the end of the culture.
 The specimens at 108, 216 and 433 µg/mL were not observed.

Table 6 Results of chromosomal aberration test (short-term treatment with S9 mix)

Treatment time (h)	S9 mix	Dose (µg/mL)	Number of cells with structural chromosomal aberrations (frequency%)										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 gaps (frequency%)	Cell growth rate (%)	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	0	0	0	0	0	0	0	0	0		
			100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			200	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.5)
6 - 18	+	108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6 - 18	+	216 †	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6 - 18	+	433 †	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6 - 18	+	865 †	100	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			100	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			200	2 (1.0)	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)	0
6 - 18	+	1730 †	100	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			100	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			200	1 (0.5)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.5)	2 (1.0)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.5)
6 - 18	+	3460 †	100	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			100	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			200	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.5)
6 - 18	+	Positive control (CPA) 6	100	27	23	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
			100	15	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			200	42 (21.0)	48 (24.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	82 (41.0)	2 (1.0)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.5)

Treatment time comprised treatment-time and recovery-time.

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

CPA: Cyclophosphamide monohydrate

†: Precipitation of the test substance was observed at the start and the end of the treatment and the end of the culture. The specimens at 108, 216 and 433 µg/mL were not observed.

Table 7 Results of chromosomal aberration test (continuous treatment)

Treatment time (h)	Dose (µg/mL)	Number of cells with structural chromosomal aberrations (frequency%)						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) 0	100	2	0	0	0	0	2	100	2	0	2
		100	1	0	1	0	0	2	100	4	0	4
		200	3 (1.5)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	4 (2.0)	200	6 (3.0)	0 (0.0)	6 (3.0)
24 - 0	108	0							94.2			
		0							96.1 (95.2)			
		0							94.4			
24 - 0	216 †	0							86.4			
		0							(90.4)			
		0							83.3			
24 - 0	433 †	0							92.2 (87.8)			
		100	1	1	0	0	0	2	100	1	0	1
		100	0	0	0	0	0	0	100	0	0	0
24 - 0	865 †	200	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)	200	1 (0.5)	0 (0.0)	1 (0.5)
		100	2	1	0	0	0	3	100	1	0	1
		100	2	0	0	0	0	2	100	1	0	1
24 - 0	1730 †	200	4 (2.0)	1 (0.5)	0 (0.0)	0 (0.0)	5 (2.5)	0 (0.0)	200	2 (1.0)	0 (0.0)	2 (1.0)
		100	3	1	0	0	0	4	100	3	0	3
		100	1	0	0	0	0	1	100	1	0	1
24 - 0	3460 †	200	4 (2.0)	1 (0.5)	0 (0.0)	0 (0.0)	5 (2.5)	1 (0.5)	200	4 (2.0)	0 (0.0)	4 (2.0)
		100	47	53	0	0	0	73	100	1	0	1
		100	52	64	0	1	0	79	100	0	0	0
24 - 0	Positive control (MMC) 0.05	200	99 (49.5)	117 (58.5)	0 (0.0)	1 (0.5)	152 (76.0)	3 (1.5)	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 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 second lines. The average of them was shown at the third line.
 MMC: Mitomycin C
 †: Precipitation of the test substance was observed at the start and the end of the treatment and the end of the culture.
 The medium was exchanged before the demecolcine solution was added 2 hours prior to the end of the culture and precipitation of the test substance was removed.
 The specimens at 108, 216 and 433 µg/mL were not observed.

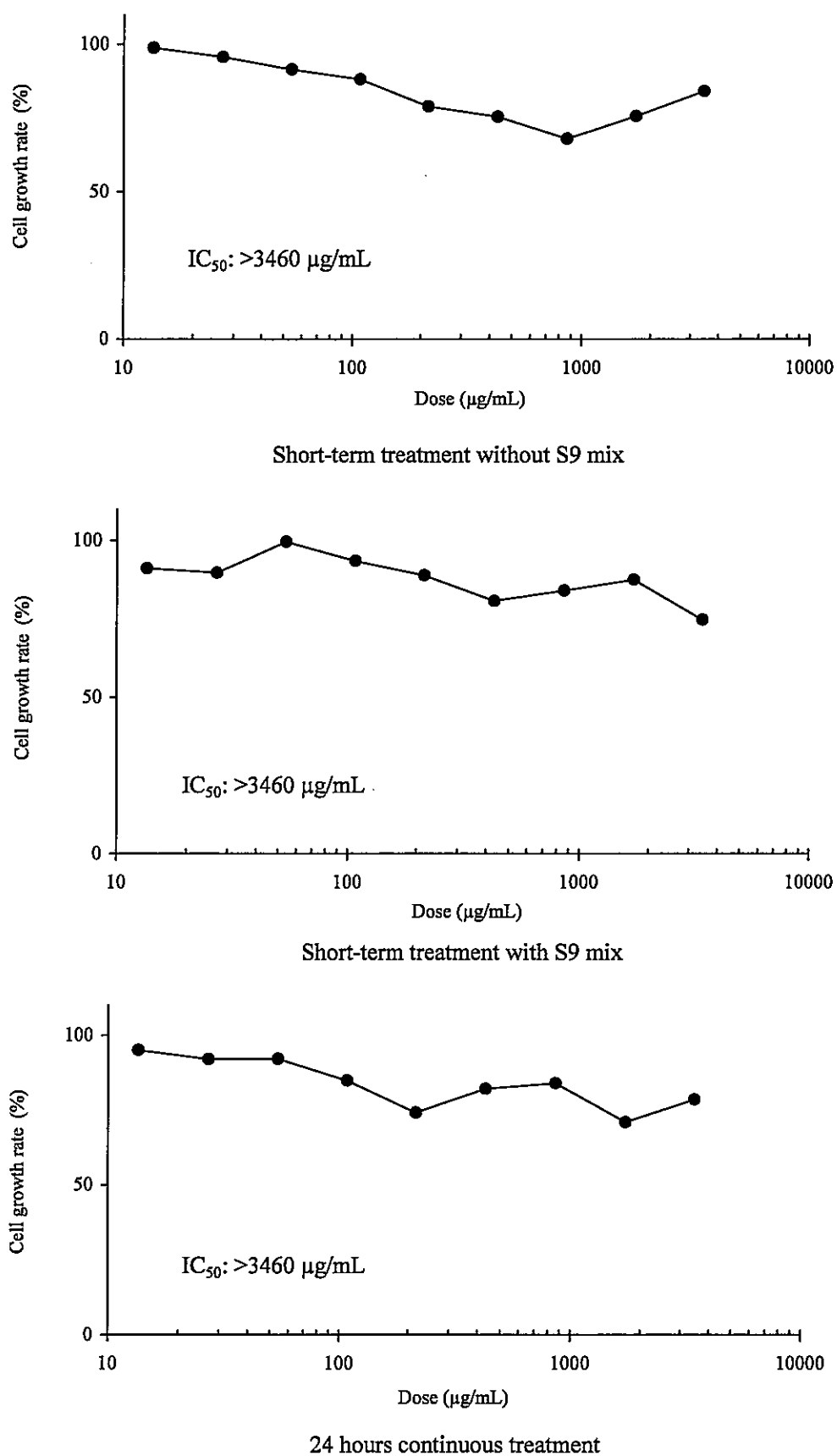


Fig. 1 Results of cell growth inhibition test of 13F-OLE

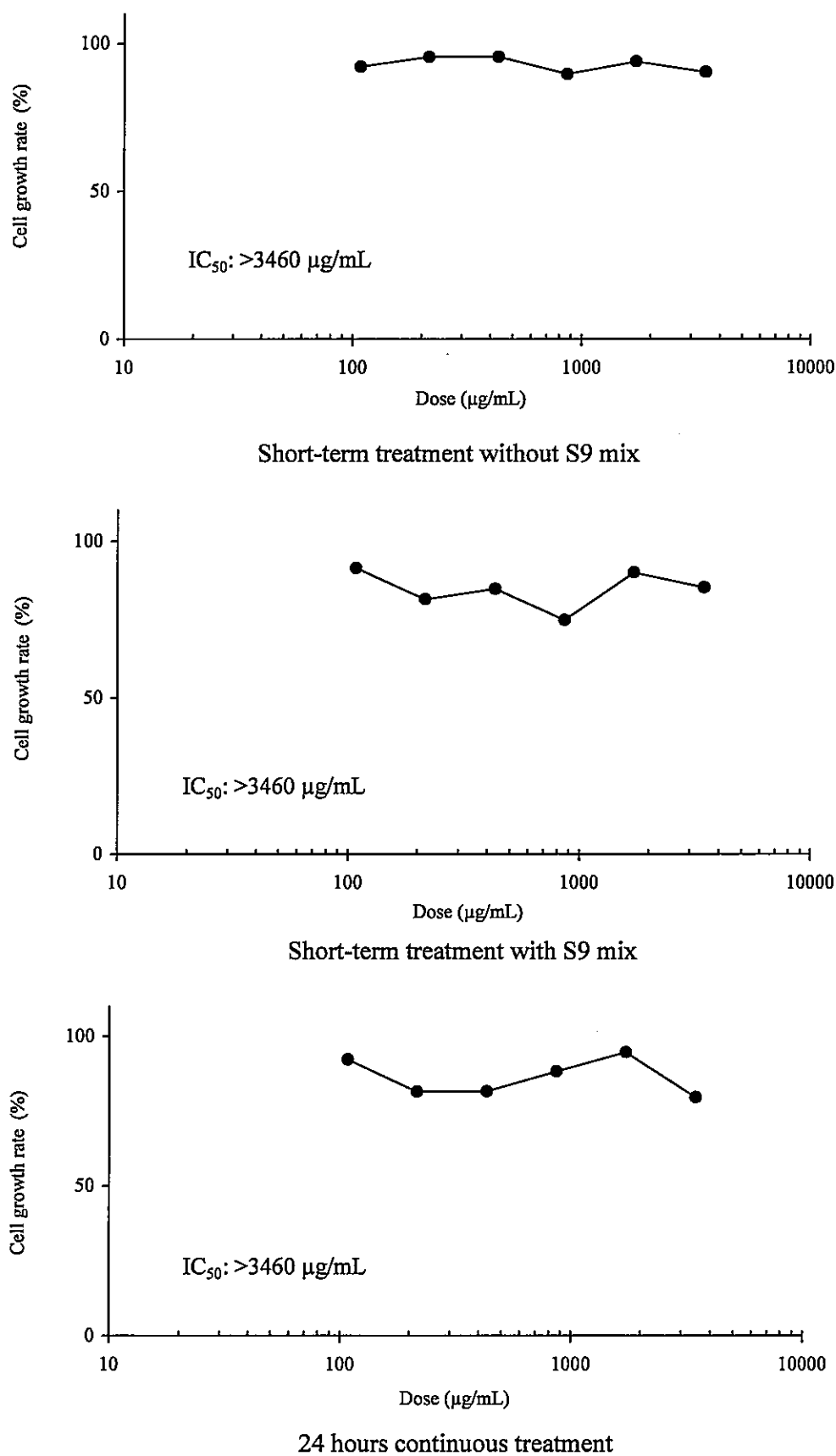


Fig. 2 Cell growth rate of the first chromosomal aberration test of 13F-OLE

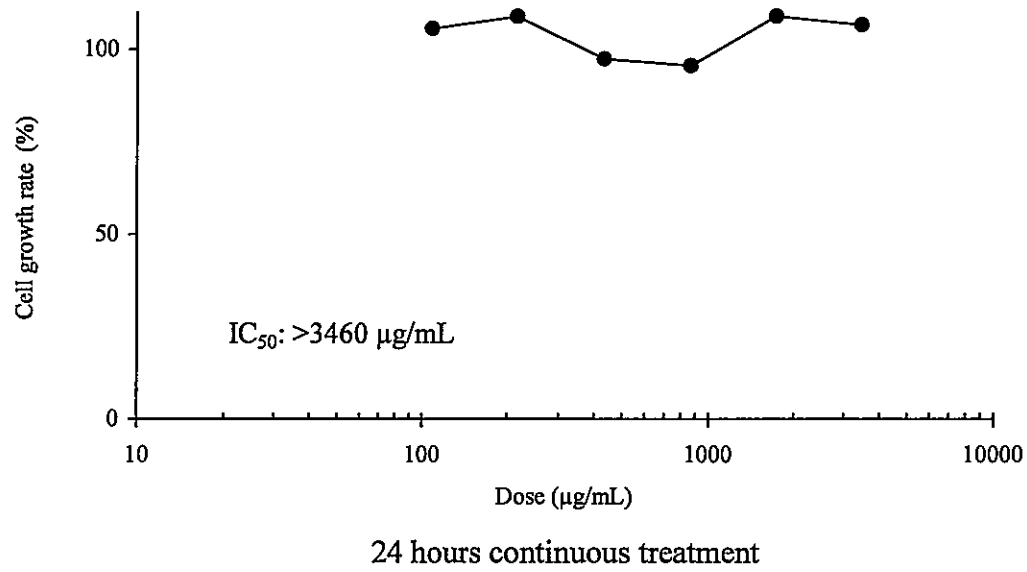


Fig. 3 Cell growth rate of the second chromosomal aberration test of 13F-OLE

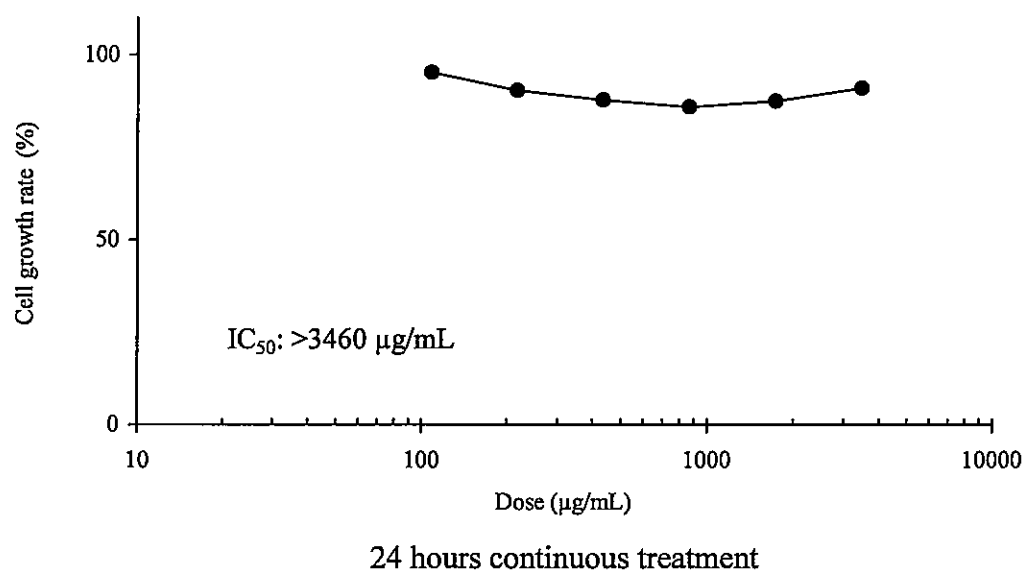


Fig. 4 Cell growth rate of the third chromosomal aberration test of 13F-OLE

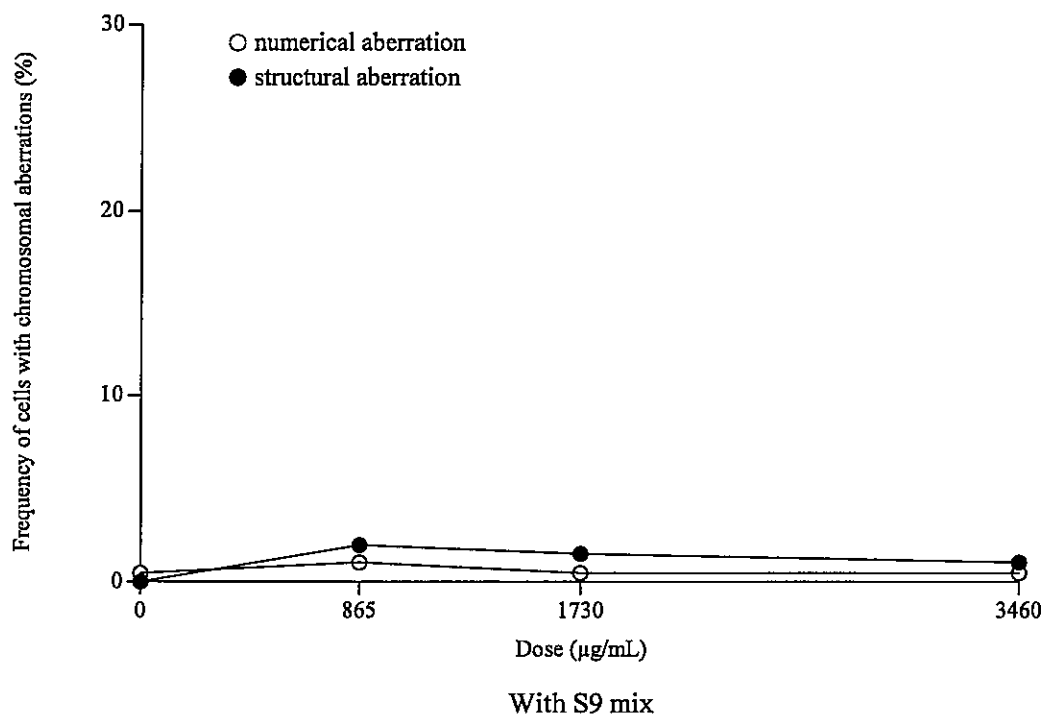
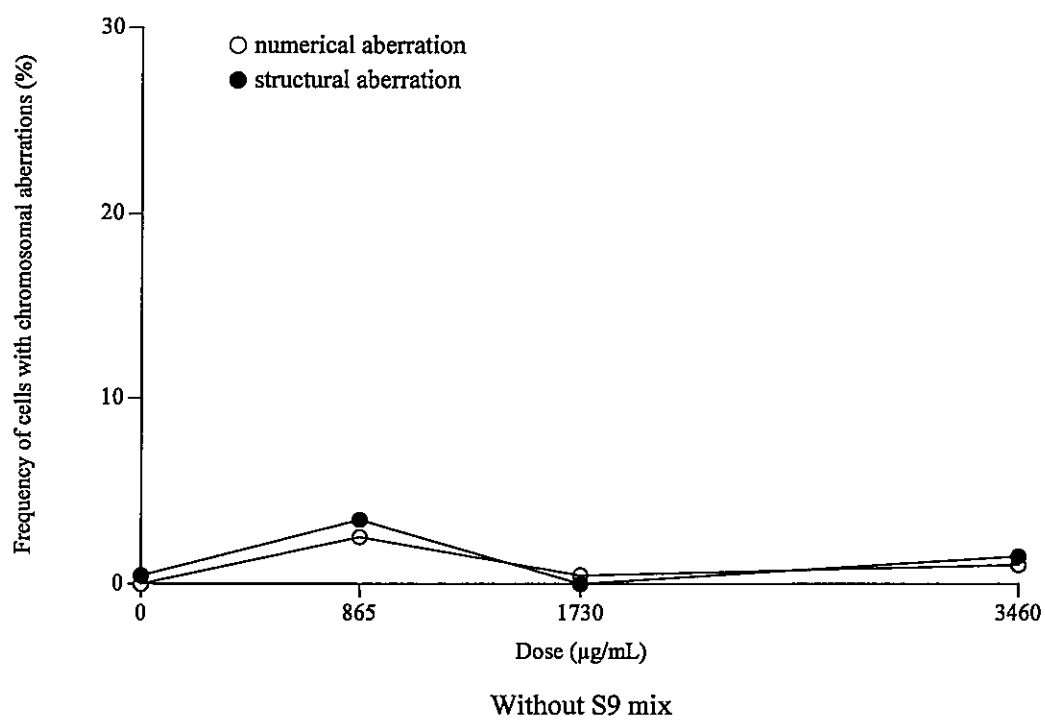


Fig. 5 Results of chromosomal aberration test in short-term treatments of 13F-OLE

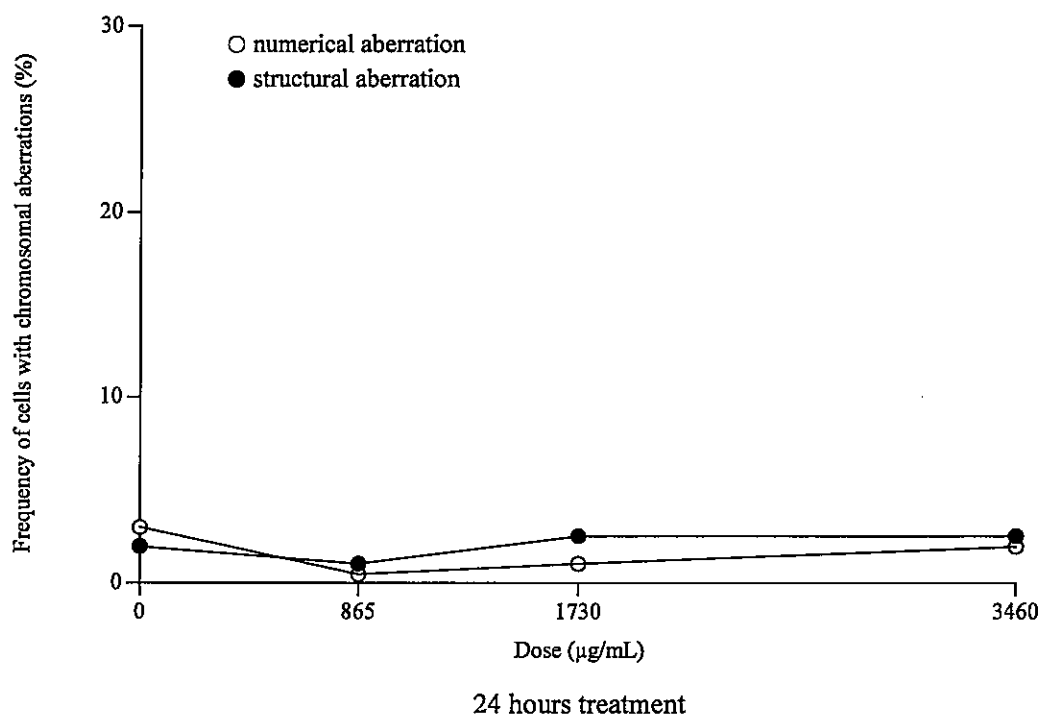


Fig. 6 Results of chromosomal aberration test in continuous treatment of 13F-OLE