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住友スリーエム株式会社　殿

試 験 報 告 書

試験報告書（２Ｌ１６２）の英訳

（試験番号：５Ｌ６２２）

T-6322.3

Sample D-1
FX-13

Chromosome
aberration test.

１９９５年１０月２３日

株式会社三菱化学安全科学研究所

STATEMENT FOR ENGLISH TRANSLATION

Kashima Laboratory

Mitsubishi Chemical Safety Institute Ltd.*

Sponsor : Sumitomo 3M Limited

Title : Chromosomal Aberration Study of Sample D-1
in Cultured Mammalian Cells

Study No. : 2L162

This study was conducted in Kashima Laboratory of Mitsubishi Chemical Safety Institute. The original report was written in Japanese. I hereby declare that this report reflects faithfully the original report as accurately as possible to my knowledge.

Translator : T. Nishitomi Date: Oct. 23, 1995

Tamotsu Nishitomi, M.S.

Senior Research Scientist

* : As from October 1, 1994, the company name has been changed.

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Submitted to:

Sumitomo 3M Limited

[REPORT]

Chromosomal Aberration Study of Sample D-1 in Cultured Mammalian Cells

(Study No. : 2L162)

September 30, 1992

MITSUBISHI-KASEI INSTITUTE OF TOXICOLOGICAL
AND ENVIRONMENTAL SCIENCES

STATEMENT

Kashima Laboratory
Mitsubishi-Kasei Institute of Toxicological
and Environmental Sciences

Sponsor : Sumitomo 3M Limited

Title : Chromosomal Aberration Study of Sample D-1
in Cultured Mammalian Cells

Study No. : 2L162

This study has been conducted in accordance with the GLP Standards applied to Industrial Chemicals of Japan.

Management: Masanobu Katoh sealed Date: September 30, 1992

QUALITY ASSURANCE STATEMENT

Kashima Laboratory
Mitsubishi-Kasei Institute of Toxicological
and Environmental Sciences

Sponsor : Sumitomo 3M Limited

Title : Chromosomal Aberration Study of Sample D-1
in Cultured Mammalian Cells

Study No. : 2L162

Study procedures were periodically inspected and the report was audited by Quality Assurance Unit. The standards of inspection adopted were in accordance with the GLP standards applied to Industrial Chemicals of Japan.

Inspection or audit	Date of inspection or audit	Date of reporting to the study director and to the management
Study procedure	April 13, 1992 April 20, 1992	April 13, 1992 April 20, 1992
Study report	July 3, 1992 September 30, 1992	July 3, 1992 September 30, 1992

Quality Assurance Unit : Yoshihiro Miura sealed Date: September 30, 1992

Title : Chromosomal Aberration Study of Sample D-1 in Cultured Mammalian Cells (Study No. 2L162)

Purpose : To assess the clastogenicity of the test substance by the chromosomal aberration test in cultured mammalian cells

Guideline : The Guidelines for Screening Toxicity Testing of Chemicals of Japan (Kampogyo No. 700, Yakuhatsu No. 1039, 61 Kikyoku No. 1014, 1986)

GLP : The GLP Standards applied to Industrial Chemicals of Japan (Kampogyo No. 39, Yakuhatsu No. 229, 59 Kikyoku No. 85, 1984)

Sponsor : Sumitomo 3M Limited

Testing facility : Kashima Laboratory
Mitsubishi-Kasei Institute of Toxicological and Environmental Sciences
14 Sunayama, Hasaki-machi, Kashima-gun, Ibaraki

Study Director : Tamotsu Nishitomi

Other Contributors : Akihiko Kido, Miyuki Tanaka

Study date : (Initiation of the study) April 10, 1992
(Submission of the final report) September 30, 1992

Unforeseeable circumstance that may have affected on the test results and deviation from the protocol have not occurred.

Retention of records :
All data, documents, the protocol and the final report will be retained in the safekeeping facility of Kashima Laboratory for 10 years after the submission of the final report. Further retention will be discussed with the sponsor.

Report of Results of Chromosomal Aberration Test in Cultured Mammalian cells

1. General Items

Name of the new chemical substance (IUPAC nomenclature)	2-[*N*-ethyl-*N*-perfruoloalkyl(C=1～8)sulfonylamino]ethylacrylate				
Other name	Sample D-1	Physico-chemical properties of the new substance	Molecular weight		625
Structural formula or rational formula (or outline of manufacturing method, in case both are unknown)			Appearance at ordinary temperature		liquid
$C_nF_{2n+1}SO_2N(C_2H_5)-CH_2CH_2O-C(=O)-CH=CH_2$ n=1～8 n=8(main component) : ca. 78% n=1～7 : ca. 21%(total) (lot No. 101)			Stability		stable
			Melting point		27～42℃
			Boiling point		ca. 150 ℃ (1mmHg)
			Vapor pressure		———
			Partition coefficient		———
			Solubility		soluble in oil
Purity of the new chemical substance tested	≧ 99%		Solubility	Water	insoluble
				DMSO	insoluble
Name and concentration of impurizies	phenothyadine 60±20ppm hydroquinonemono-methylether 170±35ppm			Acetone	soluble(≧50%)
				Others	soluble in freon 1,1,3

2. Cell line and culture condition

Name of cell line	CHL／IU	Obtained from		Dai-Nippon Pharmaceutical Co., Ltd
Species	Chinese hamster	Date obtained		6/20/1989
Medium	Eagle's MEM	Manufacturer		Nissui Pharmaceutical Co., Ltd.
Serum	Calf serum, 10 %	Manufacturer (Lot)		Gibco Lab. (50K7711) Gibco Lab. (31P6615)
Doubling time	ca. 18 h	Freezing condition		in liquid nitrogen
Passage No.	21～23*	Culture condition	Container	Plastic dish
Number of chromosomes(mode)	25		Temperature	37℃
			CO_2	5 %
Remark	* Cells were frozen at passage 20			

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3. S9 Mix

(1) Source of S9 (Encircle the applicable number, and fill in the relevant entries)

1. Made in-house	Prepared on	
(2.) Purchase	Supplier	Kikkoman Corporation
	Prepared on	3/19/1992
	Purchased on	4/ 7/1992
	Lot No.	RAA-272

(2) Storage Temperature, etc. of S9

Storage temperature	Below -80℃	Name and model of storage apparatus	Deep freezer CL-60

(3) Preparation of S9 (If purchased, fill in spaces to extent possible)

Animal used		Inducing substance	
Species, Strain	Rats, Sprague-Dawley	Name	phenobarbital (PB) 5,6-benzoflavone (BF)
Sex	Male	Administration method	Intraperitoneal
Age (in weeks)	7 weeks	Administration period and amount (g/kg-b.w.)	BP 4 days 0.03~0.06 BF 1 day 0.08
Weight	192 - 227 g		

(4) Composition of S9 Mix

Constituents	Amount in 1 ml	Constituents	Amount in 1 ml
S9	0.3 ml	$NADP^+$	4 μmol
$MgCl_2$	5 μmol	NADPH	- μmol
KCl	33 μmol	Buffer (HEPES)	4 μmol
D-Glucose 6-phosphate	5 μmol	Others (deionized water)	- ml

(5) Treatment condition with S9 Mix (Encircle the applicable number, and fill in the relevant entries)

	(1.) Plate method	2. Suspension method
Amount of S9 (Final concentration)	5 %	
Amount of S9 protein (Final concentration)	1.39 mg/ml	
Treatment time	6 h	
Culture time after treatment of the test substance	18 h	
Remark		

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4. Cell Growth Inhibition Test

(1)Test condition and preparation of the test substance solution

Period of experiment		from 4/10/1992 to 4/15/1992	
		Without metabolic activation	With metabolic activation
Cell	Number of cells seeded	4 × 10^3 /ml	4 × 10^3 /ml
	Days of culture *	3 days	3 days
Plate	Form Size Manufacturer Number of plates for each concentration	Plastic dish 6 cm in diameter Becton Dickinson & Co. 2 plates	Plastic dish 6 cm in diameter Becton Dickinson & Co. 2 plates
	Amount of medium	5 ml/plate	3 ml/plate
Preparation of the test substance solution	Solvent	DMSO	DMSO
	Concentration of the original solution of the test substance	1000 mg/ml	1000 mg/ml
	Amount of the test substance	5000 mg	5000 mg
	Volume of the solvent	5 ml	5 ml
	State of the test substance (encircle the applicable one)	Dissolved, (Suspended) Others( )	Dissolved, (Suspended) Others( )
	Time after preparetion	within 50 min.	within 65 min.
	Method of preservation	room temperature	room temperature
	Method of sterilization	not done	not done
Treatment of the cells	Amount of each test substance solution	0.025 ml/plate	0.015 ml/plate
	Period of treatment	24 and 48 h	6 h
	Amount of S9 Mix		0.5 ml/plate
Method of counting of cell number		・counting : with a hemocytometer ・fixing : with 10% formaline ・staining : with 0.1% crystal violet	・counting : with a hemocytometer ・fixing : with 10% formaline ・staining : with 0.1% crystal violet

Remark: * The initiation day of culturing was defined as 0 day.

(2)Cell growth index (Relative value when the value of the solvent-treated group is 100%)

	Concentration (μg/ml)	Cell growth index (%)
Without metabolic activation (24 h treatment)	0 (solvent)	100
	10	92
	50	61
	100 ※	22
	500 ※	34
	1000 ※	33
	2000 ※	37
	5000 ※	36
Without metabolic activation (48 h treatment)	0 (solvent)	100
	10	89
	50	78
	100 ※	29
	500 ※	14
	1000 ※	12
	2000 ※	16
	5000 ※	23
With metabolic activation	0 (solvent)	100
	10	97
	50	100
	100 ※	19
	500 ※	22
	1000 ※	12
	2000 ※	72
	5000 ※	64

※ : The test substance precipitated or floated in culture medium.

5. Chromosomal Aberration Test

(1)Test condition and preparation of the test substance solution

Period of experiment		from 4/17/1992 to 5/15/1992	
		Without metabolic activation	With metabolic activation
Cell	Number of cells seeded	4 × 10^3 /ml	4 × 10^3 /ml
	Days of culture *	3 days	3 days
Plate	Form Size Manufacturer Number of plates for each concentration	Plastic dish 6 cm in diameter Becton Dickinson & Co. 2 plates	Plastic dish 6 cm in diameter Becton Dickinson & Co. 2 plates
	Amount of medium	5 ml/plate	3 ml/plate
Preparation of of the test substance solution	Solvent	DMSO	DMSO
	Concentration of the original solution of the test substance	20 mg/ml	20 mg/ml
	Amount of the test substance	100 mg	100 mg
	Volume of the solvent	5 ml	5 ml
	State of the test substance (encircle the applicable one)	Dissolved, (Suspended) Others( )	Dissolved, (Suspended) Others( )
	Time after preparetion	within 45 min.	within 35 min.
	Method of preservation	room temperature	room temperature
	Method of sterilization	not done	not done
Treatment of the cells	Amount of each test substance solution	0.025 ml/plate	0.015 ml/plate
	Period of treatment	24 and 48 h	6 h
	Amount of S9 Mix		0.5 ml/plate
Mitotic inhibitor	Name	Colcemid	Colcemid
	Concentration	Final conc. 0.1 μg/ml	Final conc. 0.1 μg/ml
	Period of treatment	2 h	2 h

Remark: * The initiation day of culturing was defined as 0 day.

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(2)Result
Table 1, 2 show the results.

(3)Judgement of the result

Judgement (Encircle one)	Positive	(Negative)

Reason for judgement:

The test substance did not increase the cells with structural chromosomal aberrations and polyploid cells with or without metabolic activation.

These results led to the conclusion that the test substance did not have clastogenic potential.

(4) Referential matters

1. The test substance was insoluble in water and DMSO and was soluble in acetone. But the test substance in DMSO was dispersed in culture medium better than that in acetone. Therefore DMSO was selected for solvent of the test substance.

2. In the cell growth inhibition test, 50% inhibition dose of cell growth ($TCID_{50}$) was 60 $\mu g/m\ell$ in 24-hour treatment, 73 $\mu g/m\ell$ in 48-hour treatment without metabolic activation and 71 $\mu g/m\ell$ with metabolic activation. In all treatment groups, cell growth index at 100 $\mu g/m\ell$ or more did not increase or slightly increased and this concentration coincide with that beginning to precipitate. So it was considered that the test substance was saturated at 100 $\mu g/m\ell$ or more (Fig. 1, 2). From these results, the highest concentration in chromosomal aberration test was set at 100 $\mu g/m\ell$ with or without metabolic activation.

3. In chromosomal aberration test, the test substance precipitated or floated in culture medium at 100 $\mu g/m\ell$

4. Data were statistically analysed by the χ^2-test against the negative control. Structual aberrant cells only with gaps were excluded from this analysis.

6. Others

Testing facility	Name	Kashima Laboratory, Mitsubishi-Kasei Institute of Toxicological and Environmental Sciences
	Address	14 Sunayama, Hasaki-machi, Kashima-gun, Ibaraki Tel.0479(46)2871
Study director	Name title	Tamotu Nishitomi signed & sealed Senior Research Scientist
Test dates	from 4/10/1992 to 9/30/1992	

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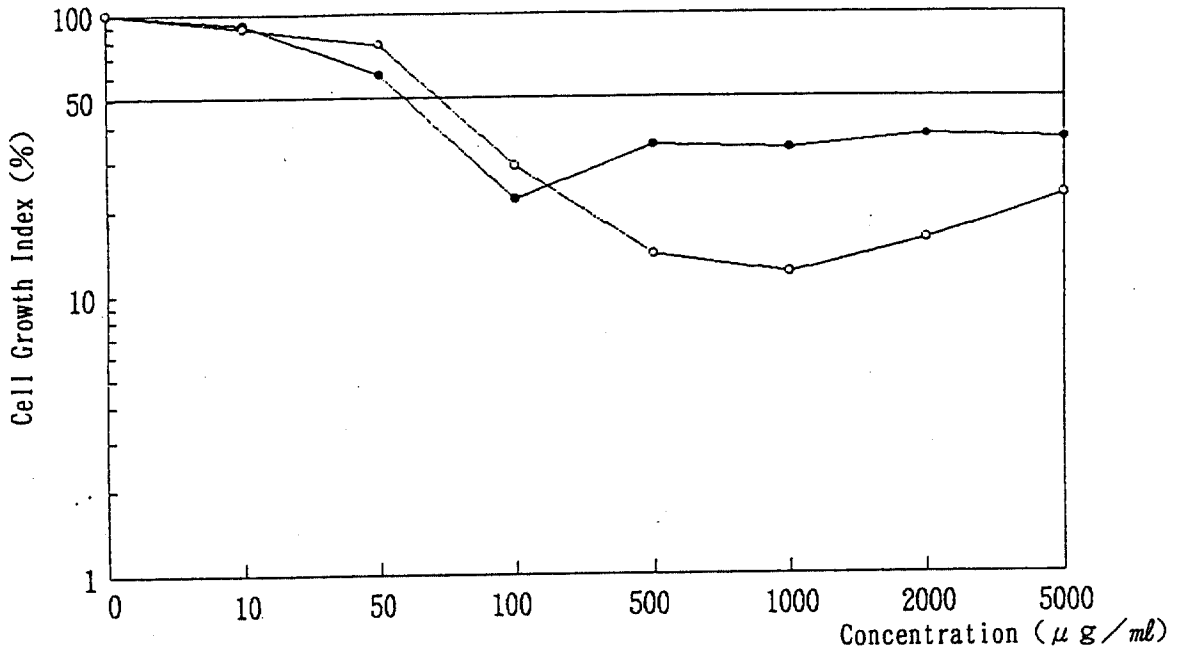
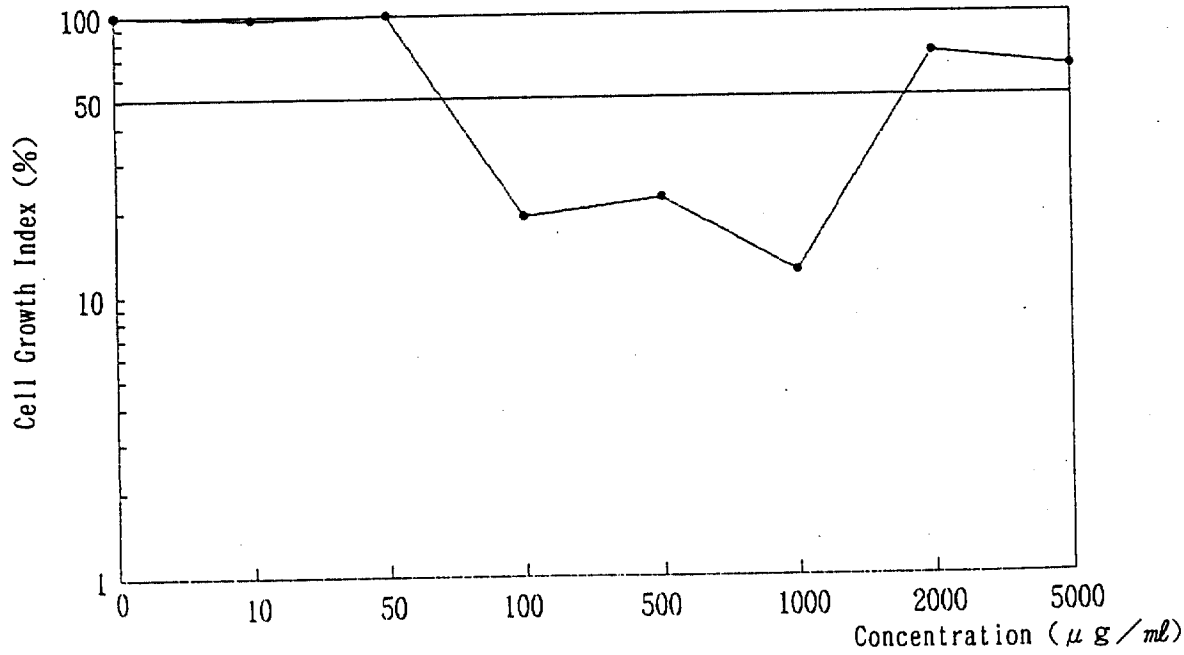


● 24-hour treatment ○ 48-hour treatment

Fig. 1 Cell growth index of Sample D-1
(without metabolic activation)



● With S9 mix (6-hour treatment and 18-hour recovery)

Fig. 2 Cell growth index of Sample D-1
(with metabolic activation)

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TABLE 1 RESULTS OF THE CHROMOSOMAL ABERRATION STUDY OF Sample D-1 IN THE DIRECT ASSAY

Test substance : Sample D-1

Treatment group	Exposure time (h)	Dose levels (μg/ml)	No. of cells analysed	Polyploids (%)	Judgement**	No. of cells with chromosomal structural aberrations *(%)								
						Gaps	Chromatid type		Chromosomal type		Others	Total		Judgement**
						g	ctb	cte	csb	cse		-g	+g	
Negative control	24	0	100	0	/	0	1	1	0	0	0	2	2	/
			100	0		0	0	0	1	0	0	1	1	
			200	0(0.0)		0(0.0)	1(0.5)	1(0.5)	1(0.5)	0(0.0)	0(0.0)	3(1.5)	3(1.5)	
(DMSO)	48	0	100	0	/	0	0	0	0	0	0	0	0	/
			100	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)	
Test substance	24	25	100	1	−	0	1	0	1	0	0	2	2	−
			100	0		0	1	0	0	0	0	1	1	
			200	1(0.5)		0(0.0)	2(1.0)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	3(1.5)	3(1.5)	
		50	100	0	−	0	0	0	1	0	0	1	1	−
			100	0		0	0	0	0	0	0	0	0	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	1(0.5)	1(0.5)	
		# 100	100	1	−	0	0	0	1	0	0	1	1	−
			100	0		0	0	0	1	0	0	1	1	
			200	1(0.5)		0(0.0)	0(0.0)	0(0.0)	2(1.0)	0(0.0)	0(0.0)	2(1.0)	2(1.0)	
	48	25	100	1	−	0	0	1	0	0	0	1	1	−
			100	1		0	0	0	0	0	0	0	0	
			200	2(1.0)		0(0.0)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	1(0.5)	
		50	100	1	−	1	0	0	1	0	0	1	2	−
			100	0		0	0	0	1	0	0	1	1	
			200	1(0.5)		1(0.5)	0(0.0)	0(0.0)	2(1.0)	0(0.0)	0(0.0)	2(1.0)	3(1.5)	
		# 100	100	1	−	0	0	0	0	0	0	0	0	−
			100	0		0	0	0	1	0	0	1	1	
			200	1(0.5)		0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	1(0.5)	1(0.5)	
Positive control	24	0.03	100	0	−	0	12	12	1	0	0	23	23	+++
			100	0		2	20	8	1	0	0	27	28	
			200	0(0.0)		2(1.0)	32(16.0)	20(10.0)	2(1.0)	0(0.0)	0(0.0)	50(25.0)	51(25.5)	
(MMC)	48	0.03	100	0	−	2	7	19	7	0	4	32	34	+++
			100	0		0	16	34	1	1	5	49	49	
			200	0(0.0)		2(1.0)	23(11.5)	53(26.5)	8(4.0)	1(0.5)	9(4.5)	81(40.5)	83(41.5)	

* ctb : chromatid break, cte : chromatid exchange, csb : chromosome break, cse : chromosome exchange, others : fragmentation etc. (except pulvalization)

** Aberrant cells(-g) were statistically compared with the control by the χ 2-test; +++($p<0.001$)

\# The test substance precipitated or floated in culture medium when it was added.

TABLE 2 RESULTS OF THE CHROMOSOMAL ABERRATION STUDY OF Sample D-1 IN THE METABOLIC ACTIVATION ASSAY

Test substance : Sample D-1

Treatment group	With or without S9 Mix	Dose levels (μg/ml)	No. of cells analysed	Polyploids (%)	Polyploids Judgement**	No. of cells with chromosomal structural aberrations *(%) Gaps g	Chromatid type ctb	Chromatid type cte	Chromosomal type csb	Chromosomal type cse	Others	Total -g	Total +g	Judgement**
Negative control	−	0	100	0	/	0	0	0	0	0	0	0	0	/
			100	0		0	0	0	1	1	0	2	2	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	1(0.5)	1(0.5)	0(0.0)	2(1.0)	2(1.0)	
(DMSO)	+	0	100	0	/	0	0	0	0	0	0	0	0	/
			100	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)	
Test substance	−	25	100	0	−	0	0	0	1	0	0	1	1	−
			100	0		0	0	0	0	0	0	0	0	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	1(0.5)	1(0.5)	
		50	100	0	−	0	1	0	0	0	0	1	1	−
			100	0		1	0	0	0	0	0	0	1	
			200	0(0.0)		1(0.5)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	2(1.0)	
		# 100	100	0	−	1	0	0	0	0	0	0	1	−
			100	0		0	0	0	0	0	0	0	0	
			200	0(0.0)		1(0.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	
	+	25	100	0	−	0	0	0	0	0	0	0	0	−
			100	0		0	0	0	1	0	0	1	1	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	1(0.5)	1(0.5)	
		50	100	0	−	0	0	0	1	0	0	1	1	−
			100	0		0	0	0	0	0	0	0	0	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	1(0.5)	1(0.5)	
		# 100	100	0	−	1	0	1	1	0	0	2	3	−
			100	0		0	0	0	1	0	0	1	1	
			200	0(0.0)		1(0.5)	0(0.0)	1(0.5)	2(1.0)	0(0.0)	0(0.0)	3(1.5)	4(2.0)	
Positive control	−	20	100	1	−	0	0	0	0	0	0	0	0	−
			100	0		0	0	1	0	0	0	1	1	
			200	1(0.5)		0(0.0)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	1(0.5)	
(BP)	+	20	100	0	−	0	12	21	0	0	44	73	73	+++
			100	1		6	15	27	0	0	36	69	71	
			200	1(0.5)		6(3.0)	27(13.5)	48(24.0)	0(0.0)	0(0.0)	80(40.0)	142(71.0)	144(72.0)	

* ctb : chromatid break, cte : chromatid exchange, csb : chromosome break, cse : chromosome exchange, others : fragmentation etc. (except pulvalization)

** Aberrant cells(-g) were statistically compared with the control by the χ 2-test; +++($p<0.001$)

\# The test substance precipitated or floated in culture medium when it was added.

Cells were treated with S9 (5% of final conc.) or without S9 for 6 hours and recovered for 18 hours.

Appendix : List of Control Reagents

Negative Control(Vehicle)

Reagent Name	Abbreviation	Lot No.	Supplier
dimethylsulfoxide	DMSO	206N1084	Kanto Chemical Co. Inc.

Positive Control

Reagent Name	Abbreviation	Lot No.	Supplier
mitomycin C	MMC	719AAB	Kyowa Hakko Kogyo Co., Ltd.
benzo[*a*]pyrene	BP	AX01	Tokyo Kasei Kogyo Co., Ltd.