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Evaluation of the Genetic Toxicity of Synthetic Chemicals (XII) -*in vitro* Chromosomal Aberration Assay with 11 Chemicals in Chinese Hamster Lung Fibroblast-

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***ABSTRACT* : The validation of many synthetic chemicals that may pose a genetic hazard in our environment is of great concern at present. Since these substances are not limited to the original products, and enter the environment, they have become widespread environmental pollutants, thus leading to a variety of chemicals that possibly threaten the public health. In this respect, the regulation and evaluation of the chemical hazard play a very important role to environment and human health. The clastogenicity of 11 synthetic chemicals was evaluated in Chinese hamster lung (CHL) fibroblast *in vitro*. Benzoyl chloride (CAS No. 98-88-4) induced chromosomal aberrations with statistical significance at the concentration of 31-123 μg/ml and 43 μg/ml in the absence and presence of S-9 metabolic activation system, respectively. 2-Propyn-1-ol (CAS No. 107-19-7) and 2-Phenoxy ethanol (CAS No. 122-99-6) revealed clastogenicity only at the highest concentration in the presence of S-9 mixture. However, 1-naphthol (CAS No. 90-15-3) which is one of the most cytotoxic chemical among 11 chemicals tested revealed no clastogenicity both in the presence and absence of S-9 metabolic activation system. From the results of chromosomal aberration assay with 11 synthetic chemicals in CHL fibroblast *in vitro*, Benzoyl chloride (CAS No. 98-88-4), 2-Propyn-1-ol (CAS No. 107-19-7) and 2-Phenoxy ethanol (CAS No. 122-99-6) revealed positive clastogenic results in this study.**

Key words : **Genotoxicity, Clastogenicity, *in vitro* Chromosome Aberration, Chinese Hamster Lung Fibroblast**

Introduction

The establishment of toxicity of synthetic chemicals that may pose a genetic hazard in our environment is subjects of great concern at present (WHO, 1971) because there are many synthetic chemicals used in chemical reaction processes in industry.

Generally, the mechanism of carcinogenicity, induction of DNA damage was ascertained by several genotoxicity assays and their potential toxicity that may consider for the human health. Several assay systems having rapidity and reliability have been introduced for this purpose, such as reversion test with bacterial gene mutation (Ames *et al.,* 1973, 1975; Maron and Ames, 1983), chromosomal aberration assay with mammalian cells (Ishidate and Odashima, 1977), micronucleus assay with rodents (Hayashi *et al.*, 1992; Schmid, 1975). These assay systems are now well used to evaluate the genotoxicity of chemicals and also frequently adopted as methods for an index of genotoxicity worldwide. Furthermore, it was well applied as a screening probe for the detection of possible carcinogenic substances in our environment.

Since the tens of thousands of man-made chemicals that have been introduced into the environment in the last few decades must also be tested for their damaging effect on DNA, the agents that cause this damage must be identified. Despite the many toxicological researches on synthetic chemicals, there are few reports on the genotoxicity of some chemicals especially well used in chemical reaction processes in industry. In this respect, our laboratory has great concern to validate the chemical hazards, and conducted the toxicity evaluations of synthetic chemicals, especially in genotoxicity (Ryu *et al.*, 1993a, 1994, 1996a,b, 1997, 1998a,b,c,d, 1999a,b, 2000, 2001 a,b,c,d, 2002a,b,c,d, 2003a,b, 2004; Kim et al, 2001; Tice *et al*, 2000).

In this study, we aim to elucidate the calstogenicity of 11 synthetic chemicals used in chemical reaction process with CHL fibroblast *in vitro*.

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Materials and Methods

The experiment was performed as described by OECD (1993) and Ishidate and Odashima (1977) with some minor modifications (Ryu *et al.*, 1993a, 1994, 1996a,b, 1998a,b, 2001b,c,d, 2002c,d, 2003a,b, 2004) which are briefly summarized as follows.

Cell Culture

A clonal sub-line of a chinese hamster lung (CHL) fibroblast was obtained from the National Institute of Health Sciences, Tokyo, Japan. The karyotype of CHL cells consisted of 25 chromosomes. The cells had been maintained by 3-4 day passages and grown in a monolayer with Eagles minimum essential medium (EMEM, Gibco, 410-1100EA) supplemented with 10% fetal bovine serum (FBS, Gibco, 26140-020). These cells were maintained at 37°C in 5% CO_2 atmosphere.

Reagents

Trypsin-EDTA and colcemid were the products of Gibco BRL Life Tech. Inc. (Gaithersburg, USA). The test chemicals were purchased from several companies (Table 1) and dissolved in dimethylsulfoxide (DMSO), 0.5% carboxymethyl cellulose (CMC) or sterilized water as indicated in Table 3. The final concentration of DMSO and CMC was below 1% and 10%, respectively. The preparation of rat liver S-9 fraction for metabolic activation system was previously reported (Ames *et al.*, 1973; Maron and Ames, 1983). The S-9 fraction prepared was stored immediately at -80°C before use.

Determination of the 50% cell growth inhibition concentration

Test article dose levels were determined prior to the main study in a dose range-finding study performed in the absence of a rat liver S-9 activation system. For the growth inhibition assay, CHL cells were seeded at the density of 5×10^4 cells/ml into 96 well plates. Twenty-four hours after seeding, several different doses of sample were separately added and incubated for 24 hours. And then the 50% inhibition concentration (IC_{50}) values of cells were calculated by MTT assay (Mosmann, 1983).

Chromosome aberration assay

For the aberration assay, three different doses, including the IC_{50} value as a maximum dose, were prepared and separately added to 3-day-old cultures (approximately 10^5 cells/60 mm dish). In the absence of metabolic activation, cultures were treated for 24 hrs with the test article, while in the presence of metabolic activation, cells were treated for 6 hrs because of toxicity of S-9 and then maintained for 18 hrs in the fresh medium to adjust a time equivalent to about 1.5 normal cell cycle lengths. Treatment was followed by addition of medium containing colcemid at a concentration of 0.2 μg/ml. After 2 hr further incubation in the presence of colcemid, metaphase cells were harvested by centrifugation and trypsinization. The cells were swollen with hypotonic (0.075 M) KCl solution for 20 min at 37°C, and washed three times in ice-cold fixative (methanol:glacial acetic acid=3:1). After centrifugation, the fixative was removed, and cell pellet suspensions were prepared by pipetting gently. A few drop of cell pellet suspension were dropped onto precleaned glass microscope slides, and air dried. Slides were stained with 5% Giemsa buffered solution at pH 6.8 for scoring of chromosome aberrations. The number of cells with chromosomal aberrations was recorded on 200 well-spread metaphases at the magnification of 1,000 with Axioscope microscope (Karl Zeiss, FRG). The classification of aberration types referred to JEMS-MMS (1988). Breaks less than the width of a chromatid were designated as gaps in our criteria, and not included as chromosomal aberration. The incidence of polyploid and endoreduplicated cells was also recorded when these events were observed. Solvent-treated cells served as controls in this experiment.

Evaluation

CHL cells usually have less than 3.0% cells with spontaneous chromosome aberrations. Aberration frequencies, defined as aberrations observed divided by number of cells counted, were analyzed using Fishers exact test (Altman, 1993) with Dunnetts adjustment and compared with results from the solvent controls. Therefore, data from count up well-spread 200 metaphase cells were expressed as percentages, and then dose-dependent responses and the statistical significance in *p*-value will be considered as positive results in our judgement.

Results and Discussion

As one of the mechanisms of carcinogenicity, it has been widely assumed that mutation represents at least one step in carcinogenesis. The evidence supporting this

idea is that the majority of mutagens are carcinogens (McCann *et al.*, 1975) and, for at least some compounds, mutagenic potency is closely correlated with carcinogenic potency (Meselson and Russel, 1991). Moreover, mutagens and certain non-mutagenic carcinogens have also been found to induce chromosomal rearrangement (Zimmermann, 1971) which may affect carcinogenesis by altering gene expression, perhaps by allowing the activation or inactivation of cellular cancer genes (Radman *et al.*, 1982). It is well known that carcinogenicity of synthetic chemicals is the most serious problem in human health hazard.

To predict the carcinogenicity of chemicals, several short term methods have been developed (Ames *et al.*, 1973; Maron and Ames, 1983; Mersch-Sundermann *et al.*, 1991) and also been introduced for the validation of genotoxicity (Ishidate and Odashima, 1977; Radman *et al*, 1982; Hayashi *et al*, 1990, 1992; Ryu *et al.*, 1993a, 1994, 1996a,b, 1997, 1998a,b,c,d, 1999a,b, 2000, 2001a,b,c,d, 2002a,b,c,d, 2003a,b, 2004) and of anti-mutagenicity (Sato *et al.*, 1991; Ryu *et al.*, 1993b, 2001c). Cytogenetic studies on mammalian cells *in vivo* (Schmid, 1975; Hayashi *et al.*, 1990, 1992, 1994) as well as *in vitro* (Ishidate and Odashima, 1977) have also been widely used as a screening method for DNA-attacking substances. The detection and the regulation of man-made synthetic chemicals are subjects of great concern because of its close correlation between environmental contamination and human health.

Among the many synthetic chemicals used in chemical reaction processes in industry, we subjected 11 chemicals in this study. The chemical name and CAS number of test chemicals were listed in Table 1 and their uses in industry are diverse. For example, dicyclopentadiene (CAS No. 77-73-6) used as monomer, and dryer and hardener in linseed and soybean oil, and pesticide intermediate, also methacrylic acid (CAS No. 79-41-4) used as manufacture of resins and plastics. And also, *a,a*-dimethylbenzyl hydroperoxide (CAS No. 80-15-9) used as polymarization catalyst and initiator. 1-Naphthol (CAS No. 90-15-3) and p-chlorophenol (CAS No. 106-48-9) is well used in organic synthesis for synthetic perfumes and drugs, and chemical intermediate for the dyes. Benzylbutyl phthalate (CAS No. 85-68-7) used as organic intermediate and pasticizer for PVC-based flooring products, and benzoyl chloride (CAS No. 98-88-4) used for acylation, i.e. introduction of the benzoyl group into alcohols, phenols, chemical intermediate for stabilizers and pesticides, and amines and of dye intermediate. Animal studies have reported the development of tumors by skin contact and vapors may cause lung injury (Fukuda *et al.*, 1981). OSHA and IARC were classified benzoyl chloride as possible carcinogen. Also, it was mutagenic for *Salmonella typhimurium* TA98 in 5 mg/plate (Chiu *et al.*, 1978). 2-Propyn-1-ol (CAS No. 107-19-7) has been used to prevent hydrogen embrittlement of steel, as corrosion inhibitor, solvent stabilizer, soil fumigant, chemical intermediate. It was also reported that 2-propyn-1-ol induced chromosomal aberrations in CHO cells *in vitro* with and without metabolic activation, while none induced reverse mutations detectable with the *Salmonella*/mammalian microsome assay. But it did not induce an increase in micronuclei in the mouse bone-

Table 1. List of 11 synthetic chemicals for chromosome aberration assay

	Chemical Name	CAS No.	Cat. No.	Manufactured by
1.	Dicyclopentadiene	77-73-6	040-01702	W
2.	Methacrylic acid	79-41-4	M0782	S
3.	*a,a*-Dimethylbenzyl hydroperoxide	80-15-9	C0524	S
4.	Benzylbutyl phthalate	85-68-7	022-04222	W
5.	1-Naphthol	90-15-3	N1000	S
6.	Benzoyl chloride	98-88-4	B0505	S
7.	p-Chlorophenol	106-48-9	C4914	S
8.	2-Propyn-1-ol	107-19-7	168-13762	W
9.	2-Phenoxy ethanol	122-99-6	P1126	S
10.	2,4-Dicholrophenyl p-nitrophenylether	1836-75-5	144-03931	W
11.	2-Nitro-p-anisidine	96-96-8	145-01582	W

W: Wako Pure Chemical Industries, Ltd. Osaka, Japan
S: Sigma-Aldrich Korea, Seoul, Korea

marrow micronucleus assay (Blakey *et al.*, 1994). 2-Phenoxyethanol (CAS No. 122-99-6) used in fixative for perfumes or cosmetic, in organic synthesis, as bactericide in conjunction with quaternary ammonium compound, as insect repellent, have been reported that may cause central nervous system depression and kidney damage (Morton, 1990). 2,4-Dicholrophenyl p-nitrophenylether (CAS No. 1836-75-5) is also used as formerly as herbicide. Nevertheless of the diverse uses of these chemicals in industry, however, there has been few attention to validate their genotoxicity.

We used CHL cells in this experiment because it was reported no diffrences of sensitivity between CHL and CHO (Chinese hamster ovary) cells in *in vitro* chromosome aberration study (Galloway *et al*, 1997). It was also reported (Henderson *et al* 1996) that extended harvest times are not necessary for the detection of *in vitro* clastogens in regulatory cytogenetic studies except it might help to resolve an equivocal result. The 50% cell growth inhibition concentration (IC_{50}) of test articles in CHL cells are obtained in the absence and presence of metabolic activation system as shown in Table 2. 1-Naphthol (CAS No. 90-15-3), 2-propyn-1-ol (CAS No. 107-19-7) and benzoyl chloride (CAS No. 98-88-4) is highly cytotoxic having IC_{50} value as 10, 20 and 43 μg/ml in the presence of metabolic activation system, respectively. Benzylbutyl phthalate (CAS No. 85-68-7), *a,a*-dimethylbenzyl hydroperoxide (CAS No. 80-15-9) and 2-nitro-p-anisidine revealed no cytotoxicity to CHL cells both in the presence and absence of S9 mixture. The concentrations used and detailed data of chromosome aberration of 11 chemicals are summarized in Table 3. The DMSO, CMC and H_2O control revealed only 0.6 - 2.0% spontaneous aberrations depending on the treatment durations in the absence and presence of metabolic activation system in 200 metaphase of CHL cells. However, the positive controls, cyclophosphamide (10 μg/ml) as an indirect mutagen that require metabolic activation, and mitomycin C (0.1 μg/ml) as a direct-acting mutagen, induced remarkable chromosome aberrations (40.5-49.4%) in CHL cells as shown in Table 3. Benzoyl chloride (CAS No. 98-88-4) induced chromosomal aberrations with statistical significance at the concentration of 31-123 μg/ml and 43 μg/ml in the absence and presence of S-9 metabolic activation system, respectively. 2-Propyn-1-ol (CAS No. 107-19-7) and 2-Phenoxy ethanol (CAS No. 122-99-6) revealed clastogenicity only at the highest concentration in the presence of S-9 mixture.

Table 2. 50% Inhibition Concentration (IC_{50}) of 11 synthetic chemicals to Chinese hamster lung fibroblast

Chemical Name	IC_{50}(μg/ml)	
	+S9	-S9
1. Dicyclopentadiene	1,416	186
2. Methacrylic acid	1,134	1,355
3. *a,a*-Dimethylbenzyl hydroperoxide	> 5,000	> 5,000
4. Benzylbutyl phthalate	> 5,000	> 5,000
5. 1-Naphthol	10	108
6. Benzoyl chloride	43	123
7. p-Chlorophenol	194	218
8. 2-Propyn-1-ol	20	1,450
9. 2-Phenoxy ethanol	3,100	1,635
10. 2,4-Dicholrophenyl p-nitrophenylether	290	78
11. 2-Nitro-p-anisidine	> 5,000	> 5,000

However, 1-naphthol (CAS No. 90-15-3) which is one of the most cytotoxic chemical among 11 chemicals tested revealed no clastogenicity both in the presence and absence of S-9 metabolic activation system.

From the results of chromosomal aberration assay with 11 synthetic chemicals in CHL fibroblast *in vitro*, Benzoyl chloride (CAS No. 98-88-4), 2-Propyn-1-ol (CAS No. 107-19-7) and 2-Phenoxy ethanol (CAS No. 122-99-6) revealed positive clastogenic results in this study.

Recently, several next generation battery of genotoxicity for the detection of genetic damages *in vitro* and *in vivo* were introduced according to the rapid progress in toxicology combined with cellular and molecular biology (Ryu 2002e,f). Among these methods, the single cell gel electrophoresis (comet assay) which can be detected DNA damages in cell level (Singh *et al.*, 1994; Ryu *et al.*, 1997, 2001a,d; Tice *et al.*, 2000), mouse lymphoma thymidine kinase gene assay (Clive *et al.*, 1983; Sawyer *et al.*, 1985; Ryu *et al.*, 1999a), FISH (fluorescence *in situ* hybridization) (Hayashi *et al.*, 1994) and transgenic animal and cell line model as a parameter of *lac I* (Big Blue) (Kohler *et al.*, 1991; Ryu *et al.*, 1998c,d, 1999b, 2000, 2002) or *lac Z* (Muta Mouse) (Suzuki *et al.*, 1993) gene mutation are newly introduced based on cellular and molecular toxicological approaches. Also, *in vivo* supravital micronucleus assay with peripheral reticulocytes by using acridine orange fluorescent staining (Hayashi *et al.*, 1990, 1992; Ryu *et al.*, 1998b) was introduced instead of mouse bone marrow micronucleus assay. Our laboratory is now under progress these assays to evaluate and to elucidate the mechanism of genetic toxicity and/or carcinogenesis, and will be presented in near future.

Table 3. Chromosome aberration assay of 11 chemicals in Chinese hamster lung fibroblast

Test chemicals (CAS No.)	Solvent	Concen-tration (μg/ml)	Treat-ment (hr)	without (-) or with(+) S9 Mix	Aberration Frequency(%)				Total aberration (%)	Extra aberration			
					Chromatid		Chromosome						
					Br	Ex	Br	Ex		ctg	csg	poly	endo
DMSO[a]			6	-	0.6±0.5	0	0	0.1±0.2	0.6±0.6	0.9±0.6	0	0.3±0.5	0
			6	+	0.9±0.6	0.2±0.3	0	0.1±0.2	1.1±0.5	0.6±0.4	0	0.8±0.9	0
			24	-	1.4±0.2	0	0	0	1.4±0.2	1.0	0	1.5	0
DW			6	-	1.0	0	0	0	1.0	2.0	0	1.5	0
			6	+	2.0	0	0	0	2.0	2.0	0	2.5	0
			24	-	0.5	0	0	0	0.5	0.5	0	0.5	0
CMC			6	-	1.8±0.3	0	0.3±0.3	0	2.0±0.5	1.5±0.5	0.3±0.3	1.3	0
			6	+	1.3±0.3	0	0.3±0.3	0.3±0.3	1.8±0.3	1.5±0.5	0.5±0.5	1.3±0.3	0
			24	-	0	0.5±0.5	0.3±0.3	0	0.8±0.3	0.3±0.3	0.3±0.3	0	0
MMC[b]		0.1	6	-	9.4±7.4	36.1±12.6	0.5±0.5	0.2±0.3	40.5±15.8	3.3±2.3	0.5±0.6	0.3±0.5	0
		0.1	24	-	10.6±4.6	47.6±24.3	0.4±0.4	0.6±0.4	48.6±22.8	3.5±2.2	0.4±0.5	1.1±1.2	0
CP		10	6	+	7.5±2.9	52.9±36.7	0.6±0.9	0	49.4±22.6	1.4±0.9	0.3±0.3	0.6±0.5	0
Dicyclo-pentadiene (77-73-6)	CMC	186	6	-	2.5	0	1.0	0.5	2.0	2.5	0	3.0	0
		93	6	-	1.5	0.5	0	0.5	2.5	1.5	0	3.5	0
		47	6	-	2.5	1.0	0.5	0	4.0	1.0	0	2.5	0
		1,416	6	+	1.5	1.5	0	0.5	3.0	2.0	0.5	1.5	0
		708	6	+	1.5	0.5	1.0	0	3.0	0.5	0	0.5	0
		354	6	+	0.5	2.0	0	0	1.5	0.5	0	0.5	0
		186	24	-	0.5	0	1.0	0	1.5	0	0	0	0
		93	24	-	2.5	0	0	0	2.0	0	0	0.5	0
		47	24	-	0	0	0	0	0	0.5	0	1.0	0
Methacrylic acid (79-41-4)	DW	1,355	6	-	2.5	0.5	0	0	3.0	2.0	0	2.0	0
		678	6	-	2.5	0	0.5	0	3.0	1.5	0.5	2.0	0
		339	6	-	3.0	0	0	0	3.0	1.5	0.5	0.5	0
		1,134	6	+	1.5	0	0	0.5	2.0	1.5	0.5	0.5	0
		567	6	+	2.5	0	0.5	0	3.0	2.0	0	1.0	0
		284	6	+	1.0	0.5	0	0	1.5	2.0	0.5	1.5	0
		1,355	24	-	2.0	0.5	0	0.5	3.0	2.0	0.5	0.5	0
		678	24	-	0	0	0.5	0.5	1.0	0.5	0	0	0
		339	24	-	0	0	0	0	0	0	0	0.5	0
α,α-Dimethyl-benzyl-hydroper-oxide (80-15-9)	CMC	5,000	6	-	0.5	1.0	1.0	0	2.5	2.5	0.5	3.0	0
		2,500	6	-	2.0	0	1.0	0	3.5	0	1.5	4.9	0
		1,250	6	-	0.5	0	0	0	0.5	2.0	0.5	5.0	0
		5,000	6	+	1.0	0.5	0	0.5	2.0	4.0	0	1.5	0
		2,500	6	+	1.5	0	0	0	1.5	1.5	0	1.5	0
		1,250	6	+	2.5	0	0.5	0	3.0	2.0	0.5	0.5	0
		5,000	24	-	0	0	0	0	0	1.0	1.5	0.5	0
		2,500	24	-	0.5	0.5	0	0	1.0	0.5	1.0	0.5	0
		1,250	24	-	1.5	0.5	0	0	2.0	0	0	0	0

Table 3. continued

Test chemicals (CAS No.)	Solvent	Concentration (μg/ml)	Treatment (hr)	without (-) or with(+) S9 Mix	Aberration Frequency(%) Chromatid Br	Chromatid Ex	Chromosome Br	Chromosome Ex	Total aberration (%)	Extra aberration ctg	csg	poly	endo
Benzylbutyl-phthalate (85-68-7)	DMSO	5,000	6	-	1.0	0.5	0	0	2.0	2.0	0	3.5	0
		2,500	6	-	1.0	0.5	0.5	0	1.5	1.0	0	2.0	0
		1,250	6	-	0	0.5	1.0	0	1.5	2.0	0	4.0	0
		5,000	6	+	2.0	1.0	0	0	3.0	0.5	0	2.0	0
		2,500	6	+	1.5	1.0	0	0	2.5	1.5	0	0.5	0
		1,250	6	+	1.0	0.5	0	0	1.5	1.0	0	3.0	0
		5,000	24	-	1.5	0.5	0	0.5	2.5	2.5	0	1.0	0
		2,500	24	-	0	0.5	0.5	0.5	1.5	1.5	1.0	0.5	0
		1,250	24	-	1.0	0	0	0	1.0	1.0	0	0	0
1-Naphthol (90-15-3)	DMSO	26.9	6	-	1.0	2.5	0	0	3.5	0.5	0	0	0
		13.5	6	-	0	0	0	0	0	0.5	0	0	0
		6.7	6	-	0	0	0	0	0	0	0	0	0
		10	6	+	1.0	0	0.5	0.5	2.0	1.5	0	0	0
		5	6	+	2.0	0.5	0.5	1.0	3.0	2.0	0.5	0	0
		2.5	6	+	1.0	0	0.5	0.5	1.5	1.5	0.5	0.5	0
		26.9	24	-	1.0	0.5	0	0	0.5	1.5	0	0	0
		13.5	24	-	1.5	0.5	0.5	0	2.0	2.5	0	0.5	0
		6.7	24	-	1.0	0	0	0	0	0	0	1.5	0
Benzoyl chloride (98-88-4)	DMSO	123	6	-	3.5	58.5	1.0	0	33.0*	1.0	0	1.5	0
		62	6	-	6.5	57.5	1.0	0	41.5*	1.5	0.5	3.0	0
		31	6	-	2.0	10.0	0	1.0	11.0*	1.5	0	5.0	0
		43	6	+	1.5	17.0	0.5	0.5	17.0*	1.0	0.5	4.5	0.5
		22	6	+	1.5	0.5	1.0	0	3.0	0.5	0	2.5	0
		11	6	+	1.0	0.5	0	0	1.5	2.0	0	1.0	0
p-Chloro-phenol (106-48-9)	DMSO	218	6	-	1.0	0	0	0	1.0	0.5	0	1.0	0
		109	6	-	0.5	0	0	0	0.5	0	0	0.5	0
		55	6	-	0.5	0	0	0	0.5	0	0	0.5	0
		194	6	+	0.5	1.0	0	0	1.5	2.5	0	0.5	1.0
		87	6	+	1.0	0	0	0	1.0	0.5	0	0	0.5
		44	6	+	1.0	2.5	0	0	3.5	2.0	0	0	0
		218	24	-	0	0.5	0	0	0.5	2.0	0	0	0
		109	24	-	0	0	0	0	0	0.5	0	0.5	0
		55	24	-	1.5	0	0	0.5	1.0	1.5	0.5	0	0
2-Propyn-1-ol (107-19-7)	DMSO	1,450	6	-	0	2.5	1.0	0	3.5	0.5	0	8.0	0
		725	6	-	0.5	2.0	0	0	2.5	0.5	0	2.5	0
		363	6	-	3.0	1.0	0	0	4.0	2.5	0	4.0	0
		20	6	+	0.5	6.0	0	0	6.5*	0	0	0	0
		10	6	+	2.0	0.5	0.5	0	3.0	0.5	0	0	0
		5	6	+	2.0	0	0	0	2.0	0	0	1.5	0
2-Phenoxy ethanol (122-99-6)	DMSO	1,635	6	-	2.0	0	0	0	2.0	1.0	0	0	0
		816	6	-	1.0	0	0	0	1.0	0.5	0	0	0
		408	6	-	0	0	0	0	0	0	0	0	0
		3,100	6	+	3.0	9.0	0	0	12.0*	0.5	0	1.5	0
		1,550	6	+	1.5	0.5	0	0	1.5	1.0	0	0	0
		775	6	+	5.0	0.5	0	0	5.5	1.5	0	0	0

Table 3. continued

Test chemicals (CAS No.)	Solvent	Concentration (μg/ml)	Treatment (hr)	without (-) or with(+) S9 Mix	Aberration Frequency(%) Chromatid Br	Chromatid Ex	Chromosome Br	Chromosome Ex	Total aberration (%)	Extra aberration ctg	csg	poly	endo
2,4-Dicholro-phenyl p-nitrophenyl-ether (1836-75-5)	DMSO	78	6	-	0.5	0.5	0	0	1.0	2.5	0.5	0	0
		36	6	-	0.5	2.0	0	0	2.5	2.0	0	0	0
		18	6	-	0	0.5	0	0	0.5	1.5	0	0	0
		290	6	+	1.0	0.5	0	0	1.5	3.0	0	0	0
		145	6	+	0	0	0	0	0	0	0	0	0
		73	6	+	0.5	0	0	0	0.5	1.0	0.5	0	0
		78	24	-	0.5	0	0	0	0.5	0	0	0	0
		36	24	-	1.0	0	0	0	1.0	0.5	0	0	0
		18	24	-	0.5	0	0	0	0.5	1.0	0.5	0	0
2-Nitro-p-anisidine (96-96-8)	DMSO	5,000	6	-	0	0.5	0	0	0.5	0.5	0	0.5	0
		2,500	6	-	0	0	0	0	0	0.5	0	0	0
		1,250	6	-	0.5	0.5	0	0	1.0	0.5	0	0	0
		5,000	6	+	4.0	2.5	0	0	6.5	2.0	0	0	0
		2,500	6	+	1.0	0.5	0	0	1.5	0	0	0	0
		1,250	6	+	0	1.0	0	0	1.0	0	0	0	0
		5,000	24	-	0.5	0	0	0	0.5	1.0	0	0	0
		2,500	24	-	1.0	0.5	0	0	1.5	0	0	0	0
		1,250	24	-	0	0	0	0	0	0	0	0	0

* significant at $p<0.05$
Br : Breakage, Ex : Exchange, ctg : chromatid gap, csg : chromosome gap, poly : polyploid, endo : endoreduplicate
DMSO : Dimethylsulfoxide, CMC : Carboxylmethyl cellulose, CP : Cyclophosphamide, MMC : Mitomycin C
The values of solvent and positive controls are expressed as mean±S.D.
[a] : solvent, [b] : positive control

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