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Evaluation of the Genetic Toxicity of Synthetic Chemicals (VI) −*In vitro* Chromosomal Aberration Assay with 17 Chemicals in Chinese Hamster Lung Cells−

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합성화학물질들의 유전독성평가(VI) −Chinese hamster lung 세포를 이용한 17종 합성화학물질들의 염색체 이상 시험−

류재천※, 김경란, 김연정, 전희경

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요 약

합성화학물질들이 환경중에 유입되어 인체에는 물론 환경생태계에 많은 영향을 미치고 있어 이들의 유해성 검증은 매우 중요한 일이라 할 수 있다. 실제 산업체에서 사용되는 수많은 화학물질들의 유전적 손상 유발유무는 더욱이 중요한 일이라 할 수 있다.

이에 따라 산업체 공정과정에서 널리 사용되는 17종의 화학물질에 대해 Chinese hamster lung (CHL) 세포를 이용한 염색체 이상 시험을 수행하여 이들이 염색체상에 구조적 이상을 유발하는지 관찰하였다. 그 결과, 본 연구에서 사용한 17종의 합성화학물질 중 2-nitroaniline (CAS No. 88-74-4)만이 대사활성화계 부재시 86.3 μg/ml의 농도에서 통계적으로 유의한 염색체 이상 유발능을 보였다. 반면 가장 높은 세포독성을 보인 1-chloroanthraquinone (CAS No. 82-44-0)은 0.8~3.0 μg/ml의 시험농도범위에서 대사활성 존재 유무와 무관하게 염색체 이상을 유발하지 않았으며, 다른 15종의 물질들 역시 본시험 적용 농도 범위에서 염색체 이상 유발능을 관찰할 수 없었다.

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

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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, 2001a, b, c, d, 2002a, b, c, d; Kim *et al.*, 2001; Heo *et al.*, 1997, Tice *et al.*, 2000).

In this study, we aim to elucidate the calstogenicity of 17 synthetic chemicals used in chemical reaction process with CHL cells *in vitro*.

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) which are briefly summarized as follows.

Cell Culture

A clonal sub-line of a chinese hamster lung (CHL) fibroblast cells 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 kindly donated and purchased from several companies and dissolved in dimethylsulfoxide (DMSO) or sterilized water as indicated in Table 2. 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% 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 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 hours with the test article, while in the presence of

metabolic activation, cells were treated for 6 hours because of toxicity of S-9 and then maintained for 18 hours 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.*, 1982, 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) and of antimutagenicity (Sato *et al.*, 1991; Ryu *et al.*, 1993b, 2001c). Cytogenetic studies on mammalian cells *in vivo* (Schmid, 1975; Hayashi *et al.*, 1982, 1990, 1992, 1994; Heo *et al.*, 1997) 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 17 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, triethyl phosphate (CAS No. 78-40-0) used as ethyla-

ting agent, catalyst, fire-retarding agent, anti-foaming agent and as a raw material to prepare insecticides such as tetraethyl pyrophospahte. And also, 2, 4-dinitrophenol (CAS No. 51-28-5) used in manufacturing of dyes, and wood preservative, insecticide, reagent and indicator. Isobutylamine (CAS No. 78-81-9) and 1-Chloroanthraquinone (CAS No. 82-44-0) is well used in organic synthesis for insecticide, and chemical intermediate for the dyes such as disperse red 9, vat brown 1 and vat orange 1, respectively. 2-nitroaniline (CAS No. 88-74-4) and 3-nitroaniline (CAS No. 99-09-2) used in dyestuff intermediate, and 4-hydroxynitrobenzene (CAS No. 100-02-7) is indicator in 0.1% alcohol solution and intermediate for the insecticide and leather preservative. 4-Vinylpyridine (CAS No. 100-43-6) used as chemical intermediate for the synthesis of poly(4-vinylpyridine). *p*-Diethylbenzene (CAS No. 105-05-5) used as a solvent. 2-Methoxyethanol (CAS No. 109-86-4) is also used as solvent for low-viscosity cellulose acetate, natural resins, some synthetic resins and some alcohol-soluble dyes, and used in dyeing leather, sealing moistureproof cellophane, enamels, and modified Karl Fischer reagent. 2-(2-aminoethylamino)ethanol (CAS No. 111-41-1) is a chemical intermediate for chelating agent, detergents, emulsifiers, gasoline stabilizers, dyeing assistants, and textile finishing compounds. Triethylamine (CAS No. 121-44-8) is catalyst for epoxy resins and polyurethane foams, and used in preparation of emusifiers for pesticides etc. Tributyl phosphate (CAS No. 126-73-8) is plasticizer for cellulose esters, lacquers, plastics, and vinyl resins, and used in aircraft hydraulic fluid, antifoam agent, and pigment grinding assistant etc. 2-Hydroxy-4-methoxybenzophenone (CAS No. 131-57-7) is ultraviolet sunscreen agent and a photostabilizer for synthetic resins. 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 of metabolic activation system as shown in Table 1. 1-Chloroanthraquinone

Table 1. 50% cell growth inhibition concentration (IC_{50}) of 17 synthetic chemicals in Chinese hamster lung cells

Chemical name	CAS No.	Cat. No.	IC_{50} (μg/ml)
1. 2, 4-Dinitrophenol	51-28-5	D-0129	115.0
2. Triethyl phosphate	78-40-0	P-0270	>1820.0
3. Isobutylamine	78-81-9	I-0095	22.8
4. 1-Chloroanthraquinone	82-44-0	C-0653	3.0
5. 2-Nitroaniline	88-74-4	N-0118	86.3
6. 3-Nitroaniline	99-09-2	N-0117	373.0
7. 4-Hydroxynitrobenzene	100-02-7	N-0220	278.2
8. 4-Vinylpyridine	100-43-6	V-0025	5.3
9. *p*-Diethylbenzene	105-05-5	D-0479	53.6
10. 2-Methoxyethanol	109-86-4	M-0111	>761.0
11. di-Isobutylamine	110-96-3	D-0614	450.7
12. 2-(2-aminoethylamino)ethanol	111-41-1	A-0299	>1041.8
13. Triethylamine	121-44-8	T-0424	661.1
14. Tri-n-butylphosphate	126-73-8	P-0266	31.0
15. 2-Hydroxy-4-methoxybenzophenone	131-57-7	H-0266	29.6
16. Mordant Black-7	3618-60-8		19.5
17. Disperse Yellow-163	71767-67-4		250.0

Table 2. Chromosome aberration assay of 17 chemicals in Chinese hamster lung cells

Test chemicals (CAS No.)	Manufactured by	Concentration (μg/ml)	without (−) or with (+) S9 mix	Aberration Frequency(%)				Total aberration (%)	Extra aberrations (%)			
				Chromatid		Chromosome						
				Br	Ex	Br	Ex		ctg	csg	poly	endo
DMSO			−	0.1±0.3	0.3±0.5	0	0	0.4±0.8	0.4±0.5	0	0	0
			+	0.1±0.3	0	0	0	0.1±0.3	1.3±0.8	0	0	0
H_2O[a]			−	0.3±0.5	0	0	0	0.3±0.5	0	0	0	0
			+	0	0.5±0.6	0	0	0.5±0.6	0.5±1.0	0	0	0
2,4-Dinitrophenol (51-28-5)	T	115.0	−	0	0	0	0	0	0	0	0	0
		57.5	−	1	0	0	0	1	0	0	0	0
		28.8	−	0	0	0	0	0	1	0	0	0
		115.0	+	1	0	0	0	1	1	0	0	0
		57.5	+	1	0	0	0	1	0	0	0	0
		28.8	+	0	0	0	0	0	0	1	0	0
Triethyl phosphate (78-40-0)	T	1820	−	0	0	0	0	0	0	0	0	0
		910	−	1	0	0	0	1	0	0	0	0
		455	−	1	0	0	0	0	0	0	0	0
		1820	+	0	0	0	0	0	1	1	0	0
		910	+	0	0	0	0	0	0	0	0	0
		455	+	0	1	0	0	1	1	0	0	0
Isobutylamine (78-81-9)	T	22.8	−	3	0	0	0	3	1	0	0	0
		11.4	−	0	0	0	0	0	0	0	0	0
		5.7	−	1	0	0	0	1	0	0	0	0
		22.8	+	1	0	0	0	1	1	0	0	0
		11.4	+	1	0	0	0	1	0	0	0	0
		5.7	+	1	0	0	0	1	0	0	0	0
1-Chloroanthraquinone[b] (82-44-0)	T	3.0	−	1	0	0	0	1	1	0	0	0
		1.5	−	2	0	0	0	2	1	0	0	0
		0.8	−	1	0	0	0	1	1	0	0	0
		3.0	+	0	0	0	0	0	1	0	0	0
		1.5	+	1	1	0	0	2	1	0	0	0
		0.8	+	1	0	0	0	1	0	0	0	0
2-Nitroaniline[b] (88-74-4)	T	86.3	−	2	5	0	0	7*	1	0	0	0
		43.2	−	2	2	0	0	4	1	0	0	0
		21.6	−	1	1	0	0	2	1	0	0	0
		86.3	+	1	1	0	0	2	0	0	0	0
		43.2	+	1	1	0	0	2	0	0	0	0
		21.6	+	0	0	0	0	0	1	0	0	0
3-Nitroaniline[b] (99-09-2)	T	373.0	−	0	0	0	0	0	0	0	0	0
		186.5	−	0	0	0	0	0	0	0	0	0
		93.3	−	0	0	0	0	0	1	0	0	0
		373.0	+	0	1	0	0	1	2	0	0	0
		186.5	+	0	0	0	0	0	1	0	0	0
		93.3	+	0	0	0	0	0	2	0	0	0
4-Hydroxynitrobenzene (100-02-7)	T	278.2	−	0	1	0	0	1	0	0	0	0
		139.1	−	0	0	0	0	0	2	0	0	0
		69.6	−	0	0	0	0	0	1	0	0	0
		278.2	+	0	0	0	0	0	2	0	0	0
		139.1	+	0	0	0	0	0	1	0	0	0
		69.6	+	0	0	0	0	0	1	0	0	0

Table 2. Continued

Test chemicals (CAS No.)	Manufactured by	Concentration (μg/ml)	without (−) or with (+) S9 mix	Aberration Frequency(%)				Total aberration (%)	Extra aberrations (%)			
				Chromatid		Chromosome						
				Br	Ex	Br	Ex		ctg	csg	poly	endo
4-Vinylpyridine (100-43-6)	T	5.3	−	0	0	0	0	0	2	0	0	0
		2.7	−	0	0	0	0	0	2	0	0	0
		1.4	−	0	0	0	0	0	1	0	0	0
		5.3	+	0	0	0	0	0	2	0	0	0
		2.7	+	0	1	0	0	1	1	0	0	0
		1.4	+	0	0	0	0	0	2	0	0	0
p-Diethylbenzene (105-05-5)	T	53.6	−	0	0	0	0	0	1	0	0	0
		26.8	−	0	0	0	0	0	0	0	0	0
		13.4	−	0	0	0	0	0	1	0	0	0
		53.6	+	0	0	0	0	0	0	0	0	0
		26.8	+	0	0	0	0	0	1	0	0	0
		13.4	+	0	0	0	0	0	1	0	0	0
2-Methoxyethanol (109-86-4)	T	761.0	−	0	0	1	0	1	1	0	0	0
		380.5	−	0	0	0	0	0	0	0	0	0
		190.3	−	0	0	0	0	0	0	0	0	0
		761.0	+	0	1	0	0	1	2	0	0	0
		380.5	+	0	0	0	0	0	0	0	0	0
		190.3	+	0	0	0	0	0	2	0	0	0
di-Isobutylamine (110-96-3)	T	450.7	−	0	0	0	0	0	0	0	0	0
		225.4	−	0	0	0	0	0	0	0	0	0
		112.7	−	0	0	0	0	0	0	0	0	0
		450.7	+	0	0	0	0	0	0	0	0	0
		225.4	+	0	1	0	0	1	1	0	0	0
		112.7	+	0	3	0	0	3	0	0	0	0
2-(2-aminoethylamino) Ethanol[a] (111-41-1)	T	1041.8	−	0	0	0	0	0	2	0	0	0
		520.9	−	0	0	0	0	0	0	0	0	0
		260.5	−	0	0	0	0	0	2	0	0	0
		1041.8	+	0	0	0	0	0	0	0	0	0
		520.9	+	0	0	0	0	0	0	0	0	0
		260.5	+	0	0	0	0	0	0	0	0	0
Triethylamine[a] (121-44-8)	T	661.1	−	0	0	0	0	0	0	0	0	0
		330.6	−	0	0	0	0	0	1	0	0	0
		165.3	−	0	0	0	1	1	1	0	0	0
		661.1	+	0	0	0	0	0	2	0	0	0
		330.6	+	0	0	0	0	0	0	0	0	0
		165.3	+	0	0	0	0	0	0	0	0	0
Tri-n-butylphosphate (126-73-8)	T	31.0	−	0	0	0	0	0	1	0	0	0
		15.5	−	0	0	0	0	0	1	0	0	0
		7.8	−	0	0	0	0	0	0	0	0	0
		31.0	+	0	0	0	0	0	1	0	0	0
		15.5	+	0	0	0	0	0	0	0	0	0
		7.8	+	0	0	0	0	0	2	0	0	0
2-Hydroxy-4-methoxybenzophenone (131-57-7)	T	29.6	−	0	0	0	0	0	0	0	0	0
		14.8	−	0	0	0	0	0	1	0	0	0
		7.4	−	0	0	0	0	0	0	0	0	0
		29.6	+	0	1	0	0	1	1	0	0	0

Table 2. *Continued*

Test chemicals (CAS No.)	Manufactured by	Concentration (μg/ml)	without (−) or with (+) S9 mix	Aberration Frequency(%) Chromatid Br	Chromatid Ex	Chromosome Br	Chromosome Ex	Total aberration (%)	Extra aberrations (%) ctg	csg	poly	endo
		14.8	+	0	0	0	0	0	2	0	0	0
		7.4	+	0	0	0	0	0	0	0	0	0
Mordant Black-7[a,b] (3618-60-8)	B	19.5	−	2	0	0	0	2	2	0	0	0
		9.8	−	0	0	0	0	0	1	0	0	0
		4.9	−	0	0	0	0	0	1	0	0	0
		19.5	+	1	1	0	0	2	1	0	0	0
		9.8	+	1	0	0	0	1	0	1	0	0
		4.9	+	0	0	0	0	0	0	0	0	0
Disperse Yellow-163[a,b] (71767-67-4)	N	250.0	−	0	1	0	0	1	1	0	0	0
		125.0	−	0	0	0	0	0	2	0	0	0
		62.5	−	0	0	0	0	0	0	0	0	0
		250.0	+	0	0	0	0	0	3	0	0	0
		125.0	+	0	1	0	0	1	0	0	0	0
		62.5	+	0	0	0	0	0	1	0	0	0
MMC	S	0.1	−	4.2±3.5	16.2±4.5	0.2±0.4	0	20.6±8.4*	3.5±1.9	0	0	0
CP	S	50	+	3.0±2.0	19.3±2.0	0.5±0.9	0	22.8±4.9*	3.8±1.1	0	0	0
B(a)P[b]	S	200	+	5.4±2.4	33.6±13.6	0	6.6±0.5	45.6±16.5*	7.2±3.5	0	0	0

* significant at $p<0.05$
Br : Breakage, Ex : Exchange, ctg : chromatid gap, csg : chromosome gap, poly : polyploid, endo : endoreduplicate
DMSO : dimethlysulfoxide, MMC : mitomycin C, B(a)P : benzo(a)pyrene, CP : Cyclophosphamide
The values of solvent and positive controls are expressed as mean±S.D.
A : Aldrich Chemical Co. Inc., WI., USA B : Bayer AG, Leverkusen, Germany
N : Nippon Kayak Co. S : Sigma Chemical Co. Ltd., Seoul, Korea.
T : Tokyo Casei Inc., Japan
[a] : solvent, H_2O [b] : positive control, B(a)P

(CAS No. 82-44-0) is the most cytotoxic having IC_{50} value as 3.0 μg/ml among 17 chemicals tested. The concentration used and detailed data of chromosome aberration of 17 chemicals are summarized in Table 2. The DMSO and H_2O control revealed only 0.1~0.5% spontaneous aberrations in the absence and presence of metabolic activation system in 200 metaphase of CHL cells. However, the positive controls, benzo(a)pyrene (200 μg/ml) and cyclophosphamide (50 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 (20.6~45.6%) in CHL cells as shown in Table 2. 2-Nitroaniline (CAS No. 88-74-4) induced chromosomal aberrations with significance at the concentration of 86.3 μg/ml in the absence of metabolic activation system. 1-Chloroanthraquinone (CAS No. 82-44-0) which is one of the most cytotoxic chemical among 17 chemicals tested revealed no clastogenicity in the range of 0.8~3.0 μg/ml both in the presence and absence of metabolic activation system.

From the results of chromosomal aberration assay with 17 synthetic chemicals in Chinese hamster lung cells *in vitro*, 2-Nitroaniline (CAS No. 88-74-4) revealed weak 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 me-

thods, 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.

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 17 synthetic chemicals was evaluated in Chinese hamster lung fibroblast cells *in vitro*. 2-Nitroaniline (CAS No. 88-74-4) induced chromosomal aberrations with statistical significance at the concentration of 86.3 μg/ml in the absence of metabolic activation system. 1-Chloroanthraquinone (CAS No. 82-44-0) which is one of the most cytotoxic chemical among 17 chemicals tested revealed no clastogenicity in the range of 0.8 ~ 3.0 μg/ml both in the presence and absence of S-9 metabolic activation system.

From the results of chromosomal aberration assay with 17 synthetic chemicals in Chinese hamster lung cells *in vitro*, 2-Nitroaniline (CAS No. 88-74-4) revealed weak positive clastogenic results in this study.

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