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Evaluation of the Genetic Toxicity of Synthetic Chemicals (X) -*In vivo* Bone Marrow Micronucleus Assay of 17 Synthetic Chemicals In Mice-

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ABSTRACT : **To validate and to estimate the chemical hazard play a very important role to environment and human health. The detection of many synthetic chemicals used in industry 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 resepct, the clastogenicity of 17 synthetic chemicals was evaluated with bone marrow micronucleus assay in mice. The positive control, mitomycin C (2 mg/kg, i.p.) revealed significant induction ratio of percentage of micronucleated polychromatic erythrocytes/1,000 polychromatic erythrocytes compared to solvent controls. The chemicals with relatively high LD_{50} value such as allyl alcohol (CAS No. 107-18-6), 2,4-pentanedione (CAS No. 123-54-6) and 4-chloro-3,5-dimethylphenol (CAS No. 88-04-0) revealed no significant induction of micronucleated polychromatic erythrocytes in mice. From this results, 17 synthetic chemicals widely used in industry have revealed no significant micronucleus induction of clastogenicity in mice in this experiment.**

Key Words : **Genotoxicity, clastogenicity, mouse bone marrow, micronucleus**

Introduction

Cytogenetic studies on mammalian cells *in vivo* as well as in vitro have been introduced as a screening method for DNA-attacking substances. It has been widely assumed that mutation represents at least one step in carcinogenesis. The evidence supporting this idea is that mutagens are carcinogens (McCann *et al.*, 1975) and, for at least some compounds, mutagenic potency is closely correlated with carcinogenic potency (Meselson and Russel, 1977). Several assay systems 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; Ryu *et al*, 1993, 1994, 1996a, 2001a, 2002a,b, 2003a), mouse lymphoma tk(+/-) gene assay with L5178Y tk+/- mouse lymphoma cells (Clive *et al.*, 1995; Sawyer *et al.*, 1985; Garriott *et al.*, 1995; Oberly and Garriott, 1996; Ryu *et al*, 1999), micronucleus assay with rodents (Hayashi *et al.*, 1982, 1983, 1990, 1992; MacGregor *et al.*, 1980, 1990; Tice *et al.*, 1990; Schmid, 1975; Ryu *et al.*, 1996b, 1998, 2001b, 2003b,c, 2004) have been introduced and also frequently adopted as methods for an index of genotoxicity in worldwide.

Among several genotoxicity assays, the micronucleus assay using immature bone marrow erythrocytes of mice has been widely used as a simple and sensitive short-term screening method *in vivo* for determining the clastogenicity of chemical substances (Schmid, 1975; Heddle, 1973). As this assay uses "whole animals", it has the merits of including such factors as absorption, distribution, and metabolism of the chemical substances in the evaluation. Although mouse bone marrow young erythrocytes (polychromatic erythrocytes) have most frequently been used as experimental material, young erythrocytes in mouse peripheral blood are increasingly being used as alternative target cells (MacGregor *et al.*, 1980, 1990; Tice *et al.*, 1990; Hayashi *et al.*, 1982, 1983, 1990, 1992, 1994).

In this study, we aim to elucidate the calstogenicity of 17 synthetic chemicals used in chemical process using *in vivo* mouse bone marrow micronucleus assay.

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Materials and Methods

The experiment was performed as described by Schmid (1975) with some minor modifications (Ryu *et al.*, 1996b, 1998, 2001b, 2003b,c, 2004) which are briefly summarized as follows.

Animals and Reagents

Outbred male mice of strain ICR were purchased from Dae-Han Laboratory Animal Co., (Eumsunggun, Korea) at 7-8 weeks of age. The mice were allowed an adaptation period of about 1 week, then randomized and subjected to the study. Mice were fed commercial pellets and tap water ad libitum throughout the acclimation and experiment periods.

Fetal bovine serum (FBS) and Giemsa stain solution were purchased from Gibco-BRL (Gaithersburg, USA). Sodium phosphate and Mitomycin C (MMC; Cat No. M0503) were purchased from Sigma (St. Louis, Mo). Carboxylmethyl cellulose sodium salt (CMC) was purchased from Showa (Japan). MMC was dissolved in physiological saline and injected once intraperitoneally at dose level of 2 mg/kg body weight. The six animals were housed for each group.

In vivo bone marrow micronucleus assay after intraperitoneal administration in mice

The test article was applied intraperitoneally or orally in three doses in volumes of 10 mL/kg. The test substance was given once, and then 24 hr interval, they killed by cervical dislocation. Normally, the tested dose range included the span from no effect up to complete halt of bone marrow proliferation. Preparation of bone marrow and staining were carried out according to the method worked out Schmid (1975). From the freshly killed animal both femora after 24 hr intraperitoneal administration were removed in toto, which means that one was cutting through pelvis and tibia. The bones were then freed from muscle by the use of gauze and fingers. With the needle of appropriate size mounted, about 1 mL of serum was pulled from the tube into a disposable plastic syringe. Then the needle (24 gauge) was inserted a few mm into the proximal part of marrow canal to flush the marrow cells. After centrifugation, the supernatant was removed, and cell pellet suspension of bone marrow cells was dropped onto glass slides, and then air dried. After fixation in methanol, slides were stained with 4% Giemsa in 1/15M sodium phosphate buffered saline (PBS, pH 6.8) for 30 min, washed with PBS, and then air dried for microscopic observation.

In scoring the preparations, micronuclei were counted in polychromatic and, separately in normochromatic erythrocytes. The rate of micronucleated cells, expressed in percentage, were based on the total of polychromatic erythrocytes present in the scored optic fields. This mode of scoring, which must always be followed where the test substance markedly influences the proliferation rate in the bone marrow, prevents a distortion of the results by the influx of peripheral blood into the damaged marrow. The scoring of micronucleated normocytes not only serves to recognize the presence of artifacts (which is rare in preparations from mouse) but provides additional interesting information on the mode of action of the test substance. Generally, an incidence of more than 1 micronucleated normocyte per thousand polychromatic erythrocytes indicates an effect on cell stages past the S-phase.

Results and Discussion

The mouse bone marrow micronucleus assay is based on the detection of the small nucleus (micronucleus) formed from chromosomal damage by chemical substances (Schmid, 1975; Heddle, 1973). The formed micronuclei remain in the cytoplasm. These micronuclei are formed by clastogenic substances and spindle poisons. When the forming function of the spindle body is obstructed, a micronucleus occurs with one to several chromosomes. Therefore, whole chromosomes containing micronuclei are observed as large size fragments rather than lagging chromosome fragments (Yamamoto and Kikuchi, 1980).

Besides, the International Agency for Research on Cancer (IARC) issues monographs containing lists of substances that cause cancer in humans (IARC, 1987). To assess the correlation between the micronucleus induction potency and carcinogenic activity, the micronucleus assay was performed as the collaborative study group for the micronucleus test by The Japanese Environmental Mutagen Society (Morita *et al.*, 1997). The experimental results of the micronucleus assay were evaluated by comparing with published data on the IARC carcinogens. The positive rates for groups 1, 2A and 2B were 68.6, 54.5 and 45.6%, respectively. After incorporating information on the structure-activity

relationship, the positive rates of the micronucleus assay become 90.5, 65.2 and 60.0% for IARC groups 1, 2A and 2B, respectively. It must be noted that the positive rates tended to be higher in carcinogens with a higher risk for human carcinogenicity. Based upon these results, it is suggested that the use of the micronucleus assay is useful as an *in vivo* short-term screening method to predict the human carcinogenicity of chemical substances.

The chemical name, CAS number, manufacurer, and LD_{50} values depending on the administration routes in mice of test chemicals were listed in Table 1 and Table 2, respectively. Among the many synthetic chemicals used in chemical reaction processes in industry, for example, 4-Chloro-2-nitroaniline (CAS No. 89-63-4), p-Nitrotoluene (CAS No. 99-99-0), 4-Nitroaniline (CAS No. 100-01-6), 4-methoxyaniline (CAS No. 104-94-9), 3,4-Dinitrotoluene (CAS No. 610-39-9), 2,4-Dichloroaniline (CAS No. 554-00-7) and 9H-Carbazole (CAS No. 86-74-8) used as a chemical intermediate for dyes and dyestuffs. Dinonyl phthalate (CAS No. 84-76-4) and allyl alcohol (CAS No. 107-18-6) used in the manufacture of plasticizers and making vinyl mixes. 1,2-Dichloro-4-nitrobenzene (CAS No. 99-54-7) is chemical intermediate for herbicides and insecticides. And also, 1,2,3-Trichloropropane (CAS No. 96-18-4), Ethylene diamine (CAS No. 107-15-3) and Ethylene glycol monoethylether (CAS No. 110-80-5) well used as solvent for casein, albumin, paint, lacquers etc. Glycidyl methacrylate (CAS No. 106-91-2) is chemical intermediate for polymers, and monomer and diluent in epoxy resin formulations. Nevertheless of the diverse and extensive uses of these chemicals in industry, however, there has been few attention to evaluate the genotoxicity of some chemicals.

In this study, we used 7-weeks-old male ICR mice and all chemicals administered orally or intraperitoneally as indicated in Table 3. The administration dose for each experiment was determined with half dose of LD_{50} value as high dose. The LD_{50} of Ethylene glycol monoethylether (CAS No. 110-80-5) was determined as 1,900 mg/kg by Lorke (1983) method in our laboratory (Table 2). The positive control, Mitomycin C (2 mg/kg, i.p.) revealed significant induction ratio of percentage of micronucleated polychromatic erythrocytes/1,000 polychromatic erythrocytes (MNPCE %/PCE) compared to solvent control. The constant range of ratio percentage of polychromatic erythrocytes/1,000 erythrocytes was also observed in all experiments as summarized in Table 3. The chemicals with relatively high LD_{50} value

Table 1. List of 17 chemicals for bone marrow micronucleus test in mice

	Chemical Name	CAS No.	Cat. No.	Manufactured by
1	4-Chloro-2-nitroaniline	89-63-4	C-0215	T
2	1,2,3-Trichloropropane	96-18-4	T-0395	T
3	1,2-Dichloro-4-nitrobenzene	99-54-7	D-0388	T
4	p-Nitrotoluene	99-99-0	N-0276	T
5	4-Nitroaniline	100-01-6	N-0119	T
6	4-methoxyaniline	104-94-9	A-0487	T
7	Glycidyl methacrylate	106-91-2	64164	F
8	Ethylene diamine	107-15-3	E-0077	T
9	Allyl alcohol	107-18-6	A-0218	T
10	2-Chloropyridine	109-09-1	C-0279	T
11	Ethylene glycol monoethylether	110-80-5	E-0047	T
12	2,4-Pentanedione	123-54-6	P-0052	T
13	3,4- Dinitrotoluene	610-39-9	D-1152	T
14	Dinonyl phthalate	84-76-4	9669	M
15	2,4-Dichloroaniline	554-00-7	11,215-1	A
16	9H-Carbazole	86-74-8	C-5132	S
17	4-Chloro-3,5-dimethylphenol	88-04-0	C-4394	S

T: Tokyo Kasei Kogyo Co. Ltd., Japan F: Fluka Chemie GmbH, Germany
M: Merck AG., Darmstadt, Germany A: Aldrich Chemical Co. Inc., WI., USA, S: Sigma Chemical Co. Ltd., USA

such as allyl alcohol (CAS No. 107-18-6), 2,4-pentanedione (CAS No. 123-54-6) and 4-chloro-3,5-dimethylphenol (CAS No. 88-04-0) revealed no significant induction of micronucleated polychromatic erythrocytes in mice. In this experiment, 17 synthetic chemicals well used in industry have revealed no significant micronucleus induction of clastogenicity in mice (Table 3).

Recently, *in vivo* supravital micronucleus assay with peripheral reticulocytes by using acridine orange fluorescent staining (Hayashi *et al.*, 1990, 1992; Heo *et al.*, 1997; Ryu *et al.*, 1998, 2001b) to distinguish

Table 2. 50% Lethal dose (LD_{50}) of 17 synthetic chemicals in mice

	Chemical Name	Route-Animal	LD_{50} (mg/kg)	Reference
1	4-Chloro-2-nitroaniline	ipr.-mus.	200	RTECS
2	1,2,3-Trichloropropane	orl.-mus.	369	RTECS
3	1,2-Dichloro-4-nitrobenzene	orl.-mus.	1384	RTECS
4	p-Nitrotoluene	orl.-mus.	1231	RTECS
5	4-Nitroaniline	ipr.-mus.	250	RTECS
6	4-methoxyaniline	ipr.-mus.	806	RTECS
7	Glycidyle methacrylate	orl.-mus.	390	RTECS
8	Ethylene diamine	ipr.-mus.	200	RTECS
9	Allyl alcohol	ipr.-mus.	60	RTECS
10	2-Chloropyridine	ipr.-mus.	130	ME
11	Ethylene glycol monoethylether	ipr.-mus.	1900	Our Data
12	2,4-Pentanedione	ipr.-mus.	60	RTECS
13	3,4- Dinitrotoluene	orl.-mus.	747	RTECS
14	Dinonyl phthalate	orl.-mus.	> 5000	RTECS
15	2,4-Dichloroaniline	ipr.-mus.	400	RTECS
16	9H-Carbazole	ipr.-mus.	200	RTECS
17	4-Chloro-3,5-dimethylphenol	ipr.-mus.	115	RTECS

RTECS : Registry of Toxic Effects of Chemical Substances
ME : Ministry of Environment, Korea

Table 3. Micronucleus Data on the bone marrow of ICR male mice i.p. or p.o. administered with 17 chemicals.

Test chemicals (CAS No.)	Dose (mg/kg)	Route	Sampling Time (hr)	MNPCE %/PCE[b] (Mean ± SD)	Ratio % of PCE/ PCE+NCE[c] (Mean ± SD)	*P*-value[a]
NC[d]	-	i.p.	24	0.11±0.07	0.49±0.02	-
	-	p.o.	24	0.14±0.08	0.50±.01	-
4-Chloro-2-nitroaniline (89-63-4)	100	i.p.	24	0.28±0.12	0.50±0.01	>0.05
	50		24	0.26±0.15	0.50±0.01	>0.05
	25		24	0.20±0.10	0.51±0.01	>0.05
1,2,3-Trichloropropane (96-18-4)	185	p.o.	24	0.15±0.14	0.50±0.02	>0.05
	92		24	0.10±0.13	0.49±0.02	>0.05
	46		24	0.18±0.10	0.47±0.04	>0.05
1,2-Dichloro-4-nitrobenzene (99-54-7)	692	p.o.	24	0.17±0.06	0.48±0.02	>0.05
	346		24	0.12±0.08	0.45±0.07	>0.05
	173		24	0.13±0.16	0.43±0.03	>0.05
p-Nitrotoluene (99-99-0)	616	p.o.	24	0.27±0.16	0.51±0.01	>0.05
	308		24	0.20±0.13	0.49±0.02	>0.05
	154		24	0.14±0.11	0.50±0.02	>0.05

Table 3. Continue

Test chemicals (CAS No.)	Dose (mg/kg)	Route	Sampling Time (hr)	MNPCE %/PCE[b] (Mean ± SD)	Ratio % of PCE/ PCE+NCE[c] (Mean ± SD)	*P*-value[a]
4-Nitroaniline (100-01-6)	125	i.p.	24	0.35±0.12	0.50±0.04	>0.05
	63		24	0.30±0.11	0.47±0.08	>0.05
	32		24	0.23±0.05	0.49±0.01	>0.05
4-methoxyaniline (104-94-9)	403	i.p.	24	0.22±0.13	0.49±0.02	>0.05
	202		24	0.18±0.13	0.48±0.01	>0.05
	101		24	0.37±0.01	0.48±0.01	>0.05
Glycidyl methacrylate (106-91-2)	195	p.o.	24	0.30±0.20	0.48±0.02	>0.05
	98		24	0.16±0.09	0.50±0.01	>0.05
	49		24	0.12±0.13	0.50±0.03	>0.05
Ethylene diamine (107-15-3)	100	i.p.	24	0.17±0.10	0.48±0.04	>0.05
	50		24	0.22±0.09	0.49±0.02	>0.05
	25		24	0.13±0.12	0.49±0.02	>0.05
Allyl alcohol (107-18-6)	30	i.p.	24	0.10±0.13	0.51±0.02	>0.05
	15		24	0.23±0.08	0.49±0.02	>0.05
	7.5		24	0.22±0.19	0.50±0.02	>0.05
2-Chloropyridine (109-09-1)	65	i.p.	24	0.13±0.05	0.49±0.01	>0.05
	32.5		24	0.08±0.12	0.50±0.02	>0.05
	16.3		24	0.17±0.15	0.51±0.01	>0.05
Ethylene glycol monoethylether (110-80-5)	1900	i.p.	24	0.07±0.01	0.47±0.02	>0.05
	950		24	0.03±0.01	0.48±0.01	>0.05
	475		24	0.07±0.01	0.48±0.01	>0.05
2,4-Pentanedione (123-54-6)	30	i.p.	24	0.18±0.13	0.49±0.02	>0.05
	15		24	0.13±0.10	0.52±0.03	>0.05
	7.5		24	0.12±0.07	.050±0.02	>0.05
3,4- Dinitrotoluene (610-39-9)	374	p.o.	24	0.07±0.01	0.50±0.03	>0.05
	187		24	0.07±0.01	0.50±0.02	>0.05
	94		24	0.28±0.01	0.48±0.02	>0.05
Dinonyl phthalate (84-76-4)	2500	p.o.	24	0.13±0.02	0.49±0.02	>0.05
	1250		24	0.05±0.01	0.47±0.01	>0.05
	625		24	0.08±0.01	0.48±0.01	>0.05
2,4-Dichloroaniline (554-00-7)	200	i.p.	24	0.15±0.01	0.49±0.01	>0.05
	100		24	0.15±0.01	0.50±0.01	>0.05
	50		24	0.10±0.01	0.49±0.02	>0.05
9H-Carbazole (86-74-8)	60	i.p.	24	0.28±0.19	0.49±0.03	>0.05
	30		24	0.22±0.10	0.48±0.02	>0.05
	15		24	0.13±0.12	0.51±0.02	>0.05
4-Chloro-3,5-dimethylphenol (88-04-0)	58	i.p.	24	0.15±0.10	0.05±0.03	>0.05
	29		24	0.12±0.08	0.49±0.01	>0.05
	15		24	0.13±0.05	0.48±0..01	>0.05
MMC[e]	2	i.p.	24	3.42±0.79*	0.50±0.01	0.0000

[a]Pairwise comparison to corresponding control, significant at $P < 0.05$

[b]MNPCE %/PCE : Percentage of Micronucleuated polychromatic erythrocytes/1,000 Polychromatic erythrocytes

[c]PCE/PCE+NCE : Ploychromatic erythrocytes/1,000 erythrocytes. [d]NC: Negative control, [e]MMC: Mitomycin C*: mean value (n=17)

immature erythrocytes in the peripheral bloods was introduced instead of mouse bone marrow micronucleus assay. This assay, briefly, the use of a DNA specific stain, acridine orange can eliminate some of the artifacts associated with using a non-DNA specific stain, Giemsa. The immature bone marrow erythrocytes enter circulation in the peripheral blood. Peripheral blood cells are obtained from the tail vein or other appropriate vessel and immediately stained supravitally. By using peripheral blood, the safety evaluation of chemical substances may be expanded from mice to rats or even to humans. As in the peripheral blood assay, the same animal can be used for several sampling and it may be possible to limit the number of animals used and the amount of each substance to be tested. As a result, more useful information about micronucleus induction could be obtained compared to the bone marrow micronucleus assay, and this method using animal blood can be applied to humans, fish, shell-fish and also to insects (Tanisho *et al.*, 1998; Hayashi *et al.*, 1998; Peace and Succop, 1999; Saotome *et al.*, 1999). Moreover, a molecular cytogenetic method, i.e., "fluorescent in site hybridization (FISH)", with centromere DNA-probes were developed (Becker *et al.*, 1990; Miller *et al.*, 1991). By this method, the presence of centromeres in micronuclei can be clearly detected, and the ability to detect differences between the micronucleus induced by clastogens or by spindle poisons became possible (Komae *et al.*, 1999).

References

Ames, B.N., Durston, W.E., Yamasaki, E. and Lee, F.D. (1973): Carcinogens are mutagens : a simple test system combining liver homogenates for activation and bacteria for detection. *Proc. Natl. Acad. Sci. USA*, **70**, 2281-2285.

Ames, B.N., McCann, J. and Yamasaki, E. (1975): Method for detecting carcinogens and mutagens with Salmonella/ mammalian-microsome mutagenicity test. *Mutation Res.*, **31**, 347-364.

Becker P, Scherthan H and Zankl H. (1990): Use of a centromere-specific DNA probe (p82H) in nonisotopic in situ hybridization for classification of micronuclei, *Genes Chromosomes Cancer*, **2**, 59-62.

Clive D, Bolcsfoldi G, Clements J, Cole J, Homna Masamitsu, Majeska J, Moore M, Muller L, Myhr B, Oberly T, Oudelhkim M-C, Rudd C, Shimada H, Sofuni T, Thybaud V and Wilcox P. (1995): Consensus agreement regarding protocol issues discussed during the mouse lymphoma workshop, *Environ. Mol. Mutagenesis*, **25**, 165-168.

Garriott ML, Casciano DA, Schechtman LM and Probst GS. (1995): International workshop on mouse lymphoma assay testing practices and data interpretations, *Environ. Mol. Mutagenesis*, **25**, 162-164

Hayashi M, Sofuni T and Ishidate M Jr. (1982): High sensitivity in micronucleus induction of mouse strain, *Mutation Res*, **105**, 252-256.

Hayashi M, Sofuni T and Ishidate M Jr. (1983): An application of acridine orange fluorescent staining to the micronucleus test, *Mutation Res.*, **120**, 241-247.

Hayashi M, Morita T, Kodama Y, Sofuni T and Ishidate M Jr. (1990): The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides, *Mutation Res.*, **245**, 245-249.

Hayashi M, Kodama Y, Awog T, Suzuki T, Asita AO and Sofuni T. (1992): The micronucleus assay using peripheral blood reticulocytes from mitomycin C- and cyclophosphamide-treated rats, *Mutation. Res.*, **278**, 209-213.

Hayashi M, Maki-Paakkanen J, Tanabe H, Honma M, Suzuki T, Matsuoka A, Mizusawa H and Sofuni T. (1994): Isolation of micronuclei from mouse blood and fluorescence in situ hybridization with a mouse centromeric DNA probe, *Mutation. Res.*, **307**, 245-251.

Hayashi M, Ueda T, Uyeno K, Wada K, Kinae N, Saotome K, Tanaka N, akai A, Sasaki YF, Asano N, Sofuni T and Ojima Y. (1998): Development of genotoxicity assay systems that use aquatic organisms, *Mutation Res.*, **399**, 125-133.

Heddle J A. (1973): A rapid *in vivo* test for chromosomal damage, *Mutation Res.*, **8**, 187-190.

Heo, M. Y., Kim, J.H. and Ryu, J.-C. (1997): Anticlastogenicity of beta-carotene and galangin using *in vivo* supravital staining micronucleus test. *Environ. Mutagens & Carcinogens*, **17**, 92-96.

IARC (International Agency for Research on Cancer). IARC monographs on the evaluation of carcinogenic risks to humans, Supplement 7, Overall Evaluation of Carcinogenicity: An updating of IARC monographs from Vols. 1 to 42, IARC, Lyon, 1987.

Ishidate, M. and Odashima, S. (1977): Chromosome test with 134 compounds on chinese hamster cells in vitro-A screening for chemical carcinogens. *Mutation Res.*, **48**, 337-354.

Komae N, Hibino Y and Sugano N. (1999): Analysis of micronuclei induced under hyperthermic conditions in human lymphocyte culture by fluorescence in situ hybridization (FISH) and spectral karyotyping (SKY) methods, Yakugaku Zasshi, **119**, 763-772.

Lorke, D. (1983): A new approach to practical acute toxicity testing. *Arch Toxicol.*, **54**, 275-287

Maron, D.M. and Ames, B.N. (1983): Revised methods for the Salmonella mutagenicity test. *Mutation Res.*, **113**, 173-215.

MacGregor JT, Wehr CM and Gould DH. (1980): Clastogen-induced micronuclei in peripheral blood erythrocytes: The

basis of an improved micronucleus test, *Environ. Mutagen.*, **2**, 509-514.

MacGregor JT, Wehr CM, Henika PR, and Shelby MD. (1990): The *in vivo* erythrocyte micronucleus test: measurement at steady state increases assay efficiency and permits integration with toxicity studies, *Fundam Appl Toxicol*, **14**(3), 513-22.

McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975): Detection of carcinogens as mutagens in the Salmonella/ microsome test : assay of 300 chemicals. *Proc Natl Acad Sci USA*, **72**, 5135-5139.

Meselson, M and Russell, K. Comparison of carcinogenesis and mutagenesis potency. Origin of Human Cancer edited by Hiatt, H.H., Watson, J.D. and Winstend, J.A. (1991): Cold Spring Harbor Laboratory, New York, pp. 1473-1481.

Miller BM, Zitzelsberger HF, Weier H-Ul G and Adler I-D. (1991): Classification of micronuclei in murine erythrocytes: Immunofluorescent staining using CREST antibodies compared to in situ hybridization with biotinylated gamma satellite DNA, *Mutagenesis*, **6**, 297-302.

Morita T, Asano N, Awogi T, Sasaki Y F, Sato S, Shimada H, Sutou S, Suzuki T, Wakata A, Sofuni T and Hayashi M. (1997): Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B): The summary report of the 6th collaborative study by CSGMT/JEMS.MMS, *Mutation Res.*, **389**, 3-122.

Oberly TJ and Garriott ML. (1996): Influence of cytotoxicity on test results in the L5178Y TK+/- mouse lymphoma assay, *Environ. Mol. Mutagenesis*, **27**, 75-78.

Peace B E and Succop P. (1999): Spontaneous micronucleus frequency and age: What are normal values? *Mutation Res.*, **425**, 225-230.

Ryu, J.-C., Lee, S., Kim, K.-R., Kim, M., Chang, I.-M. and Park, J.A. (1993): study on the clastogenicity of trichothecene mycotoxins in chinese hamster lung cells. *Korean J Toxicol.*, **9**, 13-21.

Ryu, J.-C., Lee, S., Kim, K.-R. and Park, J. (1994): Evaluation of the genetic toxicity of synthetic chemicals (I). Chromosomal aberration test on chinese hamster lung cells *in vitro. Environ Mutagens & Carcinogens*, **14**, 138-144.

Ryu, J.-C., Kim, K.-R., Ryu, E.-K., Kim, H.-J., Kwon, O.-S., Song, C.-E., Mar, W. and Chang, I.-M. (1996a): Chromosomal aberration assay of taxol and 10-deacetyl baccatin III in chinese hamster lung cells *in vitro. Environ Mutagens & Carcinogens*, **16**, 6-12.

Ryu, J.-C., Kim, K.-R., Kim, H.-J., Ryu. E.-K., Lee, S.-Y., Jung, S.-O., Youn, J.-Y., Kim, M.-H. and Kwon, O.-S. (1996b): Evaluation of the genetic toxicity of synthetic chemicals (II). a pyrethroid insecticide, fenpropathrin. *Arch Pharm Res.*, **19**, 251-257.

Ryu, J.-C., Kim, K.-R., Kim, H.-J., Myung, S.-W., Kim, G.-H., Lee, M.-J. and Chang, I.-M. (1998): Genotoxicity Study of Bojungchisup-tang, an oriental herbal decoction-in vitro chromosome aberration assay in chinese hamster lung cells and in vitro supravital-staining micronucleus assay with mouse peripheral reticulocytes, *Arch Pharm Res.*, **21**(4), 391-397.

Ryu, J.-C., Kim, K.-R. and Choi, Y.-J. (1999): in vitro mouse lymphoma thymidine kinase (tk+/-) gene forward mutation assay in mammalian cells, *Environ Mutagen & Carcinogen*, **19**(1), 7-13.

Ryu, J.-C., Kim, K.-R., Lee, S. and Park, J. (2001a): Evaluation of the genetic toxicity of synthetic chemicals (III), Chromosomal aberration assay with 28 chemicals in chinese hamster lung cells *in vitro, Environ Mutagens & Carcinogens*, **21**(1), 14-22.

Ryu, J.-C. and Park, K.Y. (2001b): Anticlastogenic effect of Baechu (Chinese cabbage) Kimchi and Buchu (leek) Kimchi in supravital staining micronucleus assay using peripheral reticulocytes of mouse, *Environ Mutagens & Carcinogens* **21**(1), 51-56.

Ryu, J.-C., Kim KR and Kim YJ. (2002a): Evaluation of the Genetic Toxicity of Synthetic Chemicals (IV) - in vitro chromosomal aberration assay with 18 chemicals in Chinese hamster lung cells-, *Environ Mutagens & Carcinogens*, **22**(3), 149-156.

Ryu, J.-C., Kim, KR, Kim YJ and Choi HY. (2002b): Evaluation of the Genetic Toxicity of Synthetic Chemicals (V) - in vitro chromosomal aberration assay with 17 chemicals in Chinese hamster lung cells-, *Environ Mutagens & Carcinogens*, **22**(4), 215-222.

Ryu, J.-C.,, Kim, KR, Kim, YJ and Jeon, HK. (2003a): Evaluation of the genetic toxicity of synthetic chemicals (VI)-in vitro chromosomal aberration assay with 17 chemicals in Chinese hamster lung cells-, *J. Environ. Toxicol.*, **18**(2), 111-120.

Ryu, J.-C. and Kim, KR. (2003b): Evaluation of the genetic toxicity of synthetic chemicals (VII)-a synthetic selective herbicide, pendimethalin-, *J. Environ. Toxicol.*, **18**(2), 121-129.

Ryu, J.-C., Kim, KR and Kim, YJ. (2003c): Evaluation of the genetic toxicity of synthetic chemicals (VIII)-*in vivo* bone marrow micronucleus assay of 8 synthetic chemicals in mice, *J. Environ. Toxicol.*, **18**(2), 137-143.

Ryu, J.-C. and Kim, YJ. (2004): Evaluation of the genetic toxicity of synthetic chemicals (IX)-a synthetic selective herbicide, Pretilachlor-, *J. Environ. Toxicol.*, **19**(1), 93-100.

Saotome K, Sofuni T and Hayashi M. (1999): A micronucleus assay in sea urchin embryos, *Mutation Res.*, **446**, 121-127.

Sawyer J, Moore MM, Clive D and Hozier J. (1985): Cytogenetic characterization of the L5178Y TK+/- 3.7.2C mouse lymphoma cell line, *Mutation Res.*, **147**, 243-253.

Schmid, W. (1975): The micronucleus test. *Mutation Res.*, **31**, 9-15.

Tanisho T, Yagi T, Iwasaki K, Shimoi K, Kinae N, Hayashi M and Sofuni T. (1998): Monitoring of coastal water contaminated with mutagens and/ or carcinogens using micronu-

cleus test in fish, *Environ. Mutagen Res.*, **20**, 1-9.
Tice RR, Erexson GL, Hilliard CJ, Huston JL, Boehm RM, Gulati D, and Shelby MD. (1990): Effect of treatment protocol and sample time on the frequencies of micronucleated polychromatic erythrocytes in mouse bone marrow and peripheral blood, *Mutagenesis*, **5**(4), 313-21.
Yamamoto KI and Kikuchi Y. A, (1980): comparison of diameters of micronuclei induced by clastogens and by spindle poisons, *Mutation Res.*, **71**, 127-131.