Evaluation of the Genetic Toxicity of Synthetic Chemicals (VII)
-A Synthetic Selective Herbicide, Pendimethalin-

Jae-Chun Ryu* and Kyung-Ran Kim

Toxicology Laboratory, Korea Institute of Science and Technology, P.O. Box 131,
Cheongryang, Seoul 130-650, Korea

합성화학물질들의 유전독성평가 (VII)
-합성 제초제인 Pendimethalin-

류 재 천*, 김 경 란

한국과학기술연구원 생체대사연구센터 독성연구실

요 약

Pendimethalin [N-(1-ethyl-propyl)-2, 6-dinitro-3, 4-xylidine, $C_{13}H_{19}N_3O_4$, M.W. = 281.3, CAS No. 40487-42-1]는 제초제의 일종으로, 본 연구에서는 박테리아 복귀 돌연변이 시험과 포유동물 세포를 이용한 염색체 이상 시험 및 마우스를 이용한 *in vivo* 소핵 시험을 수행하여 pendimethalin의 유전독성을 평가하였다.

박테리아 복귀 돌연변이 시험에서 pendimethalin은 *Salmonella typhimurium* TA98, TA1537 균주의 경우, 대사 활성계 존재와 부재시, TA100의 경우는 대사 활성계 부재시에만 313~5,000 µg/plate의 범위에서 농도의존적인 돌연변이율의 증가를 보여주었고, TA1535의 경우에는 대사 활성계 존재시 약간의 돌연변이가 증가되는 것을 관찰할 수 있었다. 그러나 대사 활성계 부재시 TA1535와 대사 활성계 존재시 TA100 균주의 경우에는 돌연변이 유발능을 관찰할 수 없었다.

한편 포유동물 세포인 Chinese hamster lung (CHL) fibroblast를 이용한 염색체 이상 시험에서 pendimethalin은 대사 활성계 존재 및 부재시 2.32~9.28 µg/ml의 농도에서 clastogenicity를 보이지 않았다. 또한 203~810 mg/kg의 pendimethalin을 구강 투여한 마우스의 골수세포를 이용한 *in vivo* 소핵 시험의 결과에서도 통계적으로 유의한 소핵 유발능을 관찰할 수 없었다.

Key words : genotoxicity, clastogenicity, pendimethalin, *Salmonella typhimurium*, chromosome aberration, micronucleus, Chinese hamster lung fibroblast

INTRODUCTION

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 agrochemicals. 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), chromoso-

---

※ To whom correspondence should be addressed.
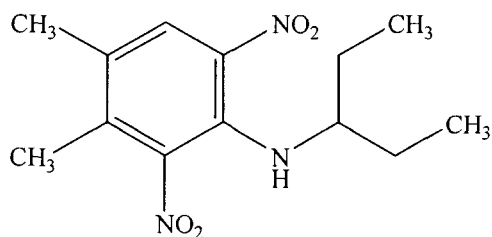Tel: +82-2-958-5070, E-mail: ryujc@kist.re.kr

Fig. 1. The chemical structure of pendimethalin.

mal aberration assay with mammalian cells (Ishidate and Odashima, 1977), micronucleus assay with rodents (Hayashi et al., 1992, 1994; 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.

Pendimethalin, [N-(1-ethylpropyl)-2, 6-dinitro-3, 4-xylidine, $C_{13}H_{19}N_3O_4$, M.W. = 281.3, CAS No. 40487-42-1], is one of well known synthetic selective herbicide. The chemical structure of pendimethalin is illustrated in Fig. 1. This chemical absorbed by the roots and leaves and affected plants die shortly after germination or following emergence from the soil with microtubule assembly inhibition activity. Our laboratory has great concern to evaluate 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).

To elucidate the genotoxicity of pendimethalin, we performed bacterial gene mutation, in vitro chromosome aberration and in vivo micronucleus assay in this study.

MATERIALS AND METHODS

Materials

Pendimethalin was orange-yellow crystal, and

stock solution was prepared freshly in dimethylsulfoxide (DMSO) before use. Eagles minimum essential medium (EMEM), RPMI-1640, 0.25% trypsin-EDTA, trypan blue, colcemid, fetal bovine serum (FBS) and horse serum were the products of Gibco BRL Life Tech. Inc. (Gaithersburg, USA). All other chemicals used were of analytical grade or the highest grade available. 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.

Ames *Salmonella* bacterial mutagenicity assay

This test performed essentially as described by Ames et al. (1973, 1975). The dose range for test chemical was determined by performing a toxicity assay using strain Salmonella typhimurium TA 100 and half-log dose intervals of the test substance up to 5 mg/plate. Strain TA 100 was chosen as the representative tester strain because of its high spontaneous reversion rate. Spontaneous revertant numbers were counted and plotted against the dose of the test chemical to produce a survival curve for the his+ genotype.

The mutagenicity assay was performed by mixing one of the tester strains which was cultured overnight, with the test substance in the presence and in the absence of S-9 mixture condition, sodium phosphate buffer added instead of S-9 mixture both in negative and positive control in test tube. Then, incubating the mixture in water bath for 30 min at 37°C and after incubation, the mixture mixed with top agar containing a minimal amount of histidine and then poured onto the surface of a r-ray sterile Petri dish (Falcon, USA) containing 25 ml of solidified bottom agar. The finished plates were incubated for 48 hr at 37°C, and revertant colonies were counted later. Negative control plates containing no added test chemical but positive control plates containing appropriate amounts of chemicals known to be active were included with each tester strain (Table 1). All platings were done in

Table 1. *Salmonella typhimurium* reversion assay of pendimethalin

Compound	Dose (μg/plate)	S-9 mixture	His+ revertants/plate (Mean±S.D.)			
			TA 98	TA 100	TA 1535	TA 1537
DMSO		−	17±2	135±7	8±2	6±1
Pendimethalin	313	−	50±6	135±7	8±2	8±5
	625	−	44±1	155±16	13±2	13±5
	1250	−	69±3	188±27	12±6	14±1
	2500	−	97±4	271±27	15±6	36±6
	5000	−	137±10	350±46	13±4	43±7
SA	1	−		613±222	456±58	
2-NF	0.2	−	60±9			
9-AA	80	−				662±151
DMSO		+	27±14	166±21	8±4	6±3
Pendimethalin	313	+	40±9	87±2	10±3	6±2
	625	+	43±7	105±15	14±6	11±5
	1250	+	51±8	115±12	12±6	18±3
	2500	+	63±15	119±10	15±4	20±1
	5000	+	73±16	209±15	16±4	32±4
2-AA	0.5	+	56±9			
2-AA	1	+		360±53		
2-AA	2	+			63±11	115±48

DMSO : dimethyl sulfoxide, SA : Sodium Azide, 2-NF : 2-Nitrofluorene, 9-AA : 9-Aminoacridine, 2-AA : 2-Aminoanthracene

triplicate, and the results were tabulated as the mean ± standard deviation for each condition. A response was considered to be positive in our criteria if there was a dose-dependent increase in revertants per plate resulting in (1) at least a doubling of the background reversion rate for strains TA 98 or TA 100 and (2) at least a tripling of the background reversion rate for strains TA 1535 or TA 1537.

In vitro chromosomal aberration assay in CHL cells

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. Chinese hamster lung (CHL) fibroblast cells had been maintained by 3~4 day passages and grown in a monolayer with EMEM supplemented with 10% FBS, 50 units/ml penicillin and 50 μg/ml streptomycin. This cells were maintained at 37°C in humidified 5% CO_2 atmosphere.

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. CHL cells were seeded at the density of 5 × 10^4 cells/ml into 96 well plates after 24 hour 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).

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. Cyclophosphamide (CP) and mitomycin C (MMC) were used as a positive control in combination with or without S-9 mixture, respectively. After 22 hr incubation, treatment was followed by addition of col-

cemid 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. 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, dose-dependent responses and the statistical significance in p-value will be considered as positive results in our judgement.

In vivo bone marrow micronucleus assay in mice after oral administration

Outbred mice of strain ICR, 7~8 weeks old, were used in this study. The mice were allowed an adaptation period for 1 week, then randomized and subjected to the study. The six animals were housed for each group. The test article was applied 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 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 1/15 M 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

It is well known that carcinogenicity of synthetic chemicals is the most serious problem in human health hazard. 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). 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. Nevertheless of the extensive use of agrochemicals, however, there has been few attention to evaluate the genotoxicity. Since these substances are not limited to the original products, and they may enter the environment and have become widespread environmental pollutants, thus leading to a variety of chemicals that possibly threaten the public health.

Since pendimethalin is one of well known synthetic herbicide, it is subjected to elucidate the genotoxicity and clastogenicity in this study. This chemical absorbed by the roots and leaves and affected plants die shortly after germination or following emergence from the soil with microtubule assembly inhibition activity. Pendimethalin also widely used to control of most annual grasses and many annual broad-leaved weeds in agricultural products including cereals, maize, carrots, potatoes etc. In rat, the major metabolic routes for pendimethalin involve hydroxylation of the 4-methyl and N-1-ethyl groups, oxidation of these alkyl groups to carboxylic acids, nitro-reduction, cyclization and conjugation (Zulian, 1990).

Ames reverse mutation assay

The mutagenic potential of pendimethalin was investigated in the *Salmonella typhimurium* microsomal activation assay. This assay detects materials that cause specific point mutations such as base-pair substitution and frameshift mutation in different *S. typhimurium* strains (TA98, TA100, TA1535 and TA1537), in the presence and in the absence of S9 mixture. Positive controls specific to each of the four tester strains resulted in the expected increases in the number of histidine revertants (Table 1). In observation of the background lawns of treated bacteria, pendimethalin was no cytotoxic at doses 5,000 μg/

plate in the absence and presence of S-9 mixture, and so, we determined as optimal maximum concentrations of pendimethalin for this assay. As summarized in Table 1, pendimethalin revealed dose-dependent mutagenic potential in 313 ~ 5,000 μg/plate of *Salmonella typhimurium* TA 98 and TA 1537 both in the absence and presence of S-9 metabolic activation system, and TA 100 only in the absence of S-9 mixture. In the TA 1535, slight increase of revertant was also observed in the presence of S-9 metabolic activation system. No mutagenic potential was observed in the TA 1535 without metabolic activation system and TA 100 in the presence of S-9 mixture. There was some different reports (EPA, 1997) between the use of hamster S-9 mixture and rat microsome in *Salmonella typhimurium*. Our data revealed same result with the use of hamster S-9 mixture. It is difficult to explain this difference at this point. In this respect, it is necessary to dissolve the species differences of pendimethalin metabolism and metabolite (s).

Cytotoxicity of pendimethalin in CHL cells

Relative survival of CHL cells following exposure to a range of concentrations of pendimethalin was determined by MTT assay. The survival percentage relative to solvent control (DMSO) was determined as a percentage of the number of cells survived after treatment without metabolic activation system. The 50% cell growth inhibition concentration (IC_{50}) of pendimethalin was determined in the absence of metabolic activation system as 9.28 μg/ml in CHL fibroblast cells (data not shown)

In vitro chromosomal aberration assay in the CHL cells

The clastogenicity of pendimethalin was assessed by its ability to cause chromosomal aberrations in cultured CHL cells. The types and frequencies of chromosomal aberrations seen in treated and control cultures for 6 hr and 24 hr pendimethalin treatment in the presence and in the absence of S-9 metabolic

Table 2. Chromosome aberration assay of pendimethalin with Chinese hamster lung fibroblast cells

Treatment			S9 Mix	Chromosome aberrations/100 cells									
				Chromatid type		Chromosome type		Total aberration (%)	Extra aberration				
Compound	Con. (µg/ml)	hr		Br	Ex	Br	Ex		ctg	csg	poly	endo	nor
D.W	—	6	+	0	0	0	0	0	2	0	0	0	98
CP	50	6	+	12	9	3	3	27	2	0	0	0	71
Pendimethalin	9.28	6	+	1	0	0	0	1	0	0	0	0	99
	4.64	6	+	1	0	0	0	1	1	0	0	0	98
	2.32	6	+	1	0	0	0	1	0	0	0	0	99
D.W	—	6	—	0	0	0	0	0	2	0	0	0	98
MMC	0.1	6	—	3	11	0	0	14	2	0	0	0	84
Pendimethalin	9.28	6	—	1	0	0	0	1	0	0	0	0	99
	4.64	6	—	1	0	0	0	1	1	0	0	0	98
	2.32	6	—	3	0	0	0	3	0	0	0	0	97

Com. : compound, Con. : concentration, Br : breakage, Ex : exchange, ctg : chromatid gap, csg : chromosome gap, poly : polyploid, endo : endoreduplicate, nor : normal, MMC : mitomycin C, CP : cyclophosphamide
Total aberration (%) : Number of chromosome aberrations/total cells (100)*100

Table 3. Micronucleus assay of pendimethalin with ICR male mice (p.o.)

Test compound	Dose (mg/kg)	No. of mice tested	Sampling time (hr)	MNPCE % (Mean ± SD)	PCE/PCE+NCE (Mean ± SD)
Corn oil	*	6	24	0.10 ± 0.11	0.49 ± 0.02
MMC	2	6	24	4.15 ± 1.26	0.50 ± 0.02
Pendimethalin	203	6	24	0.14 ± 0.11	0.45 ± 0.05
	405	6	24	0.03 ± 0.10	0.50 ± 0.04
	810	6	24	0.22 ± 0.08	0.49 ± 0.01

MNPCE : Micronucleated Polychromatic Erythrocytes/1000 Polychromatic Erythrocytes
PCE/PCE+NCE : Polychromatic Erythrocytes/1000 Erythrocytes
MMC : Mitomycin C

activation, respectively, are listed in Table 2. The concentrations for the assay was determined as 9.28, 4.64 and 2.32 µg/ml in the absence and the presence of S-9 mixture. The solvent (DMSO)-treated control was revealed only 2% of spontaneous chromosomal aberrations in 200 metaphase cells. Cyclophosphamide (50 µg/ml) used as an indirect-acting mutagen that requires metabolic activation and mitomycin (0.1 µg/ml) as a direct-acting mutagen, induced remarkable chromosomal aberrations (about 20~30%) in CHL fibroblasts. Low frequencies of breaks and fragments were seen in treated cultures and solvent controls, both with and without S-9 mixture. No statistically significant increases in the mean percentage of aberrant cells in the types of aberrations noted

between treated and solvent-control were seen both with or without S-9 mixture (Table 2). No unusual types or distributions of aberrations were evident. The mean percentage of aberrant cells without S-9 mixture ranged from 1.0 to 3.0%, compared with a mean solvent control value of 2.0%. For treatment with S-9 mixture, the mean aberrant cell percentage ranged from 1.0 to 2.0%, compared with a mean solvent control value of 2.0%. From this result, 6 hr treatment of pendimethalin was not revealed clastogenicity both in the presence and absence of S-9 mixture in this assay. Consequently, no clastogenicity of pendimethalin was observed both in the absence and presence of S-9 mixture in the concentration range of 2.32~9.28 µg/ml.

In vivo bone marrow micronucleus assay in mice after oral administration

The 50% lethal dose (LD_{50}) after oral administration in male mice was reported as 1,620 mg/kg (Tomlin, 2000), so we adopted half LD_{50} value as maximum dose. Each group was consisted of 6 mice and sampling time was 24 hour in this experiment. The positive control, mitomycin C (2 mg/kg) revealed remarkable induction of MNPCE (micronucleated polychromatic erythrocytes/1000 polychromatic erythrocytes). However, *in vivo* micronucleus assay using mouse bone marrow, pendimethalin revealed no clastogenic potential in the dose range of 203 ~ 810 mg/kg body weight of pendimethalin when administered orally compared to control level (Table 3).

Consequently, *in vitro* chromosome aberration with mammalian cells and *in vivo* bone marrow micronucleus assay revealed no clastogenic potential of pendimethalin. However, pendimethalin revealed mutagenic potential in bacterial gene mutation assay.

In mammalian cell system using Chinese hamster lung (CHL) fibroblast, no clastogenicity of pendimethalin was observed both in the absence and presence of S-9 metabolic activation system in the concentration range of 2.32 ~ 9.28 μg/ml. And also, *in vivo* bone marrow micronucleus assay, pendimethalin revealed no clastogenic potential in the dose range of 203 ~ 810 mg/kg body weight after oral administration in mice.

Consequently, *in vitro* chromosome aberration with mammalian cells and *in vivo* bone marrow micronucleus assay revealed no clastogenic potential of pendimethalin. However, pendimethalin revealed mutagenic potential in bacterial gene mutation assay.

ABSTRACT

The genotoxicity of pendimethalin [N-(1-ethyl-propyl)-2, 6-dinitro-3, 4-xylidine, $C_{13}H_{19}N_3O_4$, M.W. = 281.3, CAS No. 40487-42-1], one of selective herbicide, was evaluated in bacterial gene mutation system, chromosome aberration in mammalian cell system and *in vivo* micronucleus assay with rodent.

In bacterial gene mutation assay, pendimethalin revealed dose-dependent mutagenic potential in 313 ~ 5,000 μg/plate of *Salmonella typhimurium* TA 98 and TA 1537 both in the absence and presence of S-9 metabolic activation system, and TA 100 only in the absence of S-9 mixture. In the TA 1535, slight increase of revertant was also observed in the presence of S-9 metabolic activation system. No mutagenic potential was observed in the TA 1535 without metabolic activation system and TA 100 in the presence of S-9 mixture.

REFERENCES

Altman DG. Comparing groups-categorical data : Practical Statistics for Medical Research. Chapman & Hall, London, 1993; 229-276.

Ames BN, Durston WE, Yamasaki E and Lee FD. Carcinogens are mutagens : a simple test system combining liver homogenates for activation and bacteria for detection, Proc. Nat'l. Acad. Sci. USA 1973; 70: 2281-2285.

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

EPA, Registration Eligibility Decision, pendimethalin, List A, Case 0187, EPA 738-R-97-007, pp. 17-20, June 1997

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

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

Heo MY, Kim JH and Ryu J-C. Anticlastogenicity of beta-carotene and galangin using *in vivo* supravital staining micronucleus test, Environ. Mutagens & Carcinogens 1997; 17: 92-96.

Ishidate M and Odashima S. Chromosome test with 134

compounds on chinese hamster cells *in vitro*-A screening for chemical carcinogens, Mutation Res 1977; 48: 337-354.

JEMS-MMS Atlas of chromosome aberration by chemicals, Japanese Environmental Mutagen Society-Mammalian Mutagenicity Study Group. Tokyo 1998.

Kim Y-J, Park H-J, Kim Y, Kim M-K, Lee S-H, Jung S-H and Ryu J-C. Genotoxicity Study of sophoricoside. A constituent of *Sophora japonica*, in bacterial and mammalian cell system, Environmental Mutagens & Carcinogens 2001; 21(2): 99-105

Maron DM and Ames BN. Revised methods for the *Salmonella* mutagenicity test, Mutation Res 1983; 113: 173-215.

McCann J, Choi E, Yamasaki E and Ames BN. Detection of carcinogens as mutagens in the *Salmonella*/microsome test : assay of 300 chemicals, Proc. Nat'l. Acad. Sci. USA 1975; 72: 5135-5139.

Meselson M and Russell K. Comparison of carcinogenic and mutagenic potency. In Hiatt HH, Watson JD and Winstend JA (Eds.). Origin of Human Cancer. Cold Spring Harbor Laboratory, New York, 1991; pp. 1473-1481.

Mosmann T. Rapid colorimetric assay for cellular growth and survival : Application to proliferation and cytotoxicity assays, J. Immunol. Methods 1983; 65: 55-63.

OECD. OECD giudeline for the testing of chemicals, Documents 473, Genetic toxicology : *in vitro* mammalian cytogenetic test. Organization for Economic Cooperation and Development, Paris, France, 1993.

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

Ryu J-C, Kim S-H, Kim K-R, Lee S, Kim M, Jung H-K and Park J. A study on the antimutagenic effect of cinnamic acid derivatives in *Escherichia coli* PQ 37 (SOS Chromotest) (I), Environ. Mutagens & Carcinogens 1993b; 13: 8-17.

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

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. Evaluation of the genetic toxicity of synthetic chemicals (II). a pyrethroid insecticide, fenpropathrin, Arch. Pharm. Res. 1996a; 19: 251-257.

Ryu J-C, Kim K-R, Ryu E-K, Kim H-J, Kwon O-S,

Song C-E, Mar W and Chang I-M. Chromosomal aberration assay of taxol and 10-deacetyl baccatin III in chinese hamster lung cells *in vitro*, Environ. Mutagens & Carcinogens 1996b; 16: 6-12.

Ryu J-C, Kim H-J, Seo Y-R and Kim K-R. Single cell gel electrophoresis (comet assay) to detect DNA damage and apoptosis in cell level, Environ Mutagens & Carcinogens 1997; 17: 71-77.

Ryu J-C, Kim K-R, Kim H-J, Jung S-O, Kim M-K, Park H-S and Kim Y-H. Acute and Genetic Toxicity Study of DK 1002, a Drug Candidate for Analgesics, J. Toxicol. Pub. Health 1998a; 14(3): 427-433.

Ryu J-C, Kim K-R, Kim H-J, Myung S-W, Kim G-H, Lee M-J and Chang I-M. 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. 1998b; 21(4): 391-397.

Ryu J-C, Youn J-Y, Kim, Cho K-H and Chang I-M. Transgenic Mutagenesis assay to elucidate the mechanism of mutation in gene level, Environ. Mutagen & Carcinogen 1998c; 18(1): 1-7.

Ryu J-C, Youn J-Y, Cho K-H and Chang I-M. Mutation spectrum in *lac I* gene of transgenic Big Blue cell line following to short term exposure 4-nitroquinoline N-oxide, Environ. Mol. Mutagenesis 1998d; 31(29): 16.

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

Ryu J-C, Youn J-Y, Kim Y-J, Kwon O-S, Y-S, Kim H-T, Cho K-H and Chang I-M. Mutation spectrum of 4-nitroquinoline N-oxide in the *lac I* transgenic Big Blue Rat2 cell line, Mutation Res 1999b; 445: 127-135.

Ryu J-C, Kim Y-J, Kim H-T and Chai Y-G. Genotoxicity Assessment of atrazine in the Big Blue rat2 *lac I* transgenic cell line, Environ. Mol. Mutagenesis 2000; 35(31): 52 (No. 176).

Ryu J-C, Seo Y-R, Smith MA and Han SS. The Effect of methyl methanesulfonate (MMS)-induced excision repair on p53-dependent apoptosis in human lymphoid cells, Res. Comm. Mol. Pathol. and Pharmacol. 2001a; 109(1,2): 35-51.

Ryu J-C, Kim K-R, Lee S and Park J. 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

2001b; 21(1): 14-22.

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

Ryu J-C, Kwon O-S and Kim H-T. Optimal conditions of Single Cell Gel Electrophoresis (Comet) Assay to detect DNA single strand breaks in mouse lymphoma L5178Y cells, Environ. Mutagens & Carcinogens 2001d; 21(2): 89-94.

Ryu J-C, Kim Y-J and Chai Y-G. Mutation spectrum of 1, 2-dibromo-3-chloropropane, an endocrine disruptor, in the *lac I* tranasgenic Big Blue Rat2 fibroblast cell line, Mutagenesis 2002a; 17(4): 301-307.

Ryu J-C, Kim HT and Kim YJ. Studies on DNA single strand break of seven phthalate analogues in mouse lymphoma cells, Environ. Mutagens & Carcinogens 2002b; 22(3): 164-168.

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

Ryu J-C, Kim KR, Kim YJ and Choi HY. 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, 2002d; 22(4): 215-222.

Schmid W. The micronucleus test, Mutation Res. 1975; 31: 9-15.

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu J-C and Sasaki YF. Single cell gel/comet assay : Guidelines for *in vitro* and *in vivo* genetic toxicology testing, Environ. Mol. Mutagenesis 2000; 35(3): 206-221.

Tomlin CDS. The pesticide manual, British Crop Protection Council, 12th Ed: 2000; 714-715.

Zulian J. Study of the absoorption, excretion, metabolism, and residues in tissues in rats treated with carbon-14-labeled pendimethalin, PROWL herbicide, J. Agric. Food Chem. 1990; 38: 1743-1754.