한국환경분석학회지 제15권 (제1호) 46~54, 2012
J. of the Korea Society for Environmental Analysis

In Vitro Mammalian Chromosomal Aberration Assay with Methyl Tert-butyl Ether and Furfuryl Alcohol in Chinese Hamster Lung Cells

Soo-Jin Kim, Kyung-Taek Rim†, Cheol-Hong Lim, and Jeong-Sun Yang

Center for Chemical Safety and Health, Occupational Safety and Health Research Institute, Korea Occupational Safety and Health Agency, Daejeon, Korea

Received January 26, 2012/Accepted March 31, 2012

It was performed the *in vitro* mammalian chromosomal aberration (CA) assay to gain additional information concerning the hazards associated with the use of methyl tert-butyl ether (CAS No. 1634-04-4) and furfuryl alcohol (CAS No. 98-00-0). The assay was performed using the Chinese hamster lung fibroblast cell (CHL/IU cell), by the direct method (-S9) and by the metabolic activated method (+S9 mix).Using the direct method, the 7 dosages in a 48 hour treatment group did not show that the frequency of CA is proportion to the dosage addition. The frequency of CA is not proportion to the dosage addition for a 6 hour treatment using the metabolic activated method. From these findings, it was decided that the two chemicals do not induce chromosomal aberrations under the tested conditions.

Key words: methyl tert-butyl ether, furfuryl alcohol, chromosomal aberration, *in vitro*, Chinese hamster lung

1. Introduction

The necessity for hazard identification has increased, because the frequency of chemical exposure for workers is increasing as the chemical industries have been developed. Many chemicals that are used in industry represent current concerns since they may pose genetic hazards for humans. Also these substances have become widespread as environmental pollutants, thus leading to concerns about a variety of chemicals that possibly threaten the health of workers. In this respect, the evaluation and regulation of chemical hazards are important to human health and the working environment.

This study was conducted because insufficient information was available about the potential hazards of methyl tert-butyl ether (CAS No. 1634-04-4) and furfuryl alcohol (CAS No. 98-00-0), therefore an *in vitro* mammalian chromosomal aberration assay was performed to include in hazard identification.

Methyl tert-butyl ether (MTBE) is a colorless terpene-like odor liquid which is very soluble in ethyl alcohol and ethyl ether[1]. Its major uses are as octane booster in gasoline, manufacture of isobutene (approved by EPA)[2]. Unleaded gasoline usually contains additives for octane improvement including MTBE[3].

Furfuryl alcohol (FA, expressed as "FA" in this report) is colorless to yellow liquid which miscible with alcohol, ether, acetone, ethyl acetate, and most organic solvents with the exception of paraffinic hydrocarbons[4].

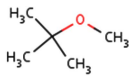
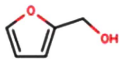
FA is used as a solvent in the manufacture in resins, as a wetting agent, a gel retarder, a diluent, a liquid propellant, in flavoring, and in foundry cores[5] may result in its release to the environment through various waste streams. It is also used as a food additive for use only as a component of adhesives[6].

The physicochemical properties of these two chemicals are represented in Table 1.

Based on collective evidence, it appears unlikely that MTBE alone induces adverse acute health effects in the general human population under common exposure conditions. In studies on animals, MTBE is "moderately"

†To whom correspondence should be addressed.
Tel: +82-42-869-0345, Fax: +82-42-863-9001, E-mail: rim3249@gmail.com

Table 1. The physicochemical properties of two chemicals in this study both MTBE and FA

Chemical Name (CAS No.)	 Methyl t-butyl ether (1634-04-4)	 Furfuryl alcohol (98-00-0)
Molecular Weight	88.15	98.10
Melting Point	-1.09E+02°C	-3.10E+01°C
Boiling Point	55.2°C	171°C
Dissociation Constant (pKa)	-3.70E+00 @ 23°C	-
log P (octanol-water)	0.94	0.28
Water Solubility	5.10E+04 mg/L @ 25°C	1.00E+06 mg/L @ 25°C
Vapor Pressure	250 mmHg @ 25°C	0.609 mmHg @ 25°C
Henry's Law Constant	5.87E-04 atm-m^3/mole @ 25°C	7.86E-08 atm-m^3/mole @ 25°C
Atmospheric OH Rate Constant	2.94E-12 cm^3/molecule-sec @ 25°C	1.04E-10 cm^3/molecule-sec @ 25°C

acutely toxic and induces mild skin and eye irritation but not sensitization. Repeated exposure in rodents affects primarily the kidney and the liver. MTBE is not genotoxic but has induced tumors in rodents primarily at high concentrations that also induce other adverse effects[7].

There is limited evidence in experimental animals for the carcinogenicity of MTBE. So, the MTBE is not classifiable as to it carcinogenicity to humans (Group 3)[8,9]. At present, no human data is available regarding potential genotoxic effects of MTBE. Limited genetic studies in rats have shown that MTBE may damage DNA and cause unscheduled DNA synthesis[9].

Occupational exposure to MTBE may occur during its production or subsequent use, particularly in gasoline through inhalation or dermal contact. The general population may be exposed to MTBE *via* inhalation of ambient air especially during refueling operations and from ingestion of ambient and drinking water[10].

FA induced symptoms of respiratory irritation, including severe bronchitis with spasms of coughing and chest pain, when used in confined spaces during hot weather[11]. Under ordinary usage in industrial plant for about 20 years has resulted in no impairment of health and it is markedly irritating & injurious to the eyes[12]. Many of these reactive chemicals such as FA in the synthetic resin systems are sensitizers, and have now been implicated in cases of occupational asthma among foundry workers[13]. Three workers exposed to FA for 15 min up to 43 mg/m^3 (11 ppm) did not report discomfort[14].

The LC_{50} value of FA in rat with inhalation exposure is 233 ppm/4 hr[15]. The LD_{50} in rat with oral exposure is 275 mg/kg fed as 2% aqueous solution[16]. The LD_{50} in rat with intraperitoneal route is 650 mg/kg[17]. The LD_{50} in rat with subcutaneous route is 85 mg/kg. The LD_{50} in mouse with oral exposure is 160 mg/kg. The LD_{50} in rabbit with dermal route is 400 mg/kg. The LD_{50} in rabbit with subcutaneous route is 650 mg/kg[18]. The LC_{50} *Daphnia magna* (Water flea) is 115 mg/L/24 hr[19].

Sister-chromatid exchanges (SCEs) in human lymphocytes were studied using the FPG technique in order to determine the cytogenetic effect of furfural and FA. The induction of SCEs was also investigated in workers occupationally exposed to these solvents that are commonly used in the manufacture of furoic resins. The results obtained from the *in vitro* treatments show that furfural increased the number of SCEs, while FA did not. In exposed workers, neither of these solvents increased the spontaneous frequency of SCEs per metaphase[20].

The national institute for occupational safety and health (NIOSH) recommended exposure limit is that 10 hr time-weighted average 10 ppm (40 mg/m^3). The 15 min short-term exposure limit (STEL) is 15 ppm (60 mg/m^3), skin[21].

The chromosomal aberration assay, conducted using mammalian cells, is frequently used to evaluate the genotoxicity of chemicals and has been adopted as an index of genotoxicity worldwide. Furthermore, it is

utilized as a screening probe for the detection of possible carcinogenic substances. Despite its increasing use, the available genotoxicity data for these two chemicals are still controversial. Therefore, we evaluated their genotoxicity using the *in vitro* mammalian chromosomal aberration assay. And this study is believed to be the first one in which chromosomal aberration of these chemicals was estimated *in vitro* with cultured Chinese hamster lung fibroblast cell line.

2. Materials and Methods

2.1. Cells and chemicals

The cultivated CHL/IU (Chinese hamster lung fibroblast) cells used in this test were obtained from the American type culture collection (CRL-1935, Koram Biotech Corp., Seoul, Korea). Cells were cultured in MEM medium (GIBCO BRL, NY, USA, Lot No. 875545, contained 10% FBS) with 5% CO_2 at 37°C, and sub-cultured every 2-4 days.

Dimethylsulfoxide (DMSO, MO, USA, Lot No. 108K0186) was used as a negative control for MTBE (Sigma, MO, USA, 98.8%, Lot No. 32596KM), and distilled water for furfuryl alcohol (Sigma, MO, USA, >98.0%, Lot No. 1253060 & 30306019) as a solvent according to results of a solubility test. Mitomycin C (MMC) (Sigma, MO, USA, Lot No. 010M0665) and cyclophosphamide (CPA) (Sigma, MO, USA, Lot No. 120M1253V) were used as positive controls in the study. For the metabolic activated system, the S9 (MOLTOX™, Maryland, USA, Lot No. 2588) was used within 6 months after manufacture.

2.2. *In vitro* mammalian chromosomal aberration test

This study was performed according to OECD guidelines for the testing of chemicals[22] and Ishidate's report[23]. For the cell proliferation suppression test, 7 dosages of each chemical (0.156, 0.3125, 0.625, 1.25, 2.5, 5 and 10 mM MTBE and FA) were use, respectively.

With direct method (24 and 48 hour treatment) not used a co-factor supplemented post-mitochondrial fraction (S9), the CHL/IU cells were cultured for 3 days from an aliquot of $2\times10^4 \sim 4\times10^4$ cells, in a 60 mm diameter plate. And with metabolic activated method (6 hour treatment),the most commonly used system is a co-factor supplemented post-mitochondrial fraction (S9) prepared from the livers of rodents treated with enzyme-inducing agents such as Aroclor 1254, or a combination of phenobarbitone and β-naphthoflavone. In the metabolic activated method, the cells were cultured using conditions identical to the direct method. Slides for observation of chromosomal samples were made from 5 mL media aliquots, with 18 hour supplementary culture, after removal of media and washing of the cell layer with 5 mL fresh media.

The main test was performed using dosages established by the cell proliferation suppression/preliminary test. After 24 and 48 hours of exposure to test chemicals, plates were treated with 0.2 μg/mL Colcemid® (GIBCO BRL, NY, USA, Lot No. 843825). After 2 hours, the metaphase cells were separated and centrifuged at 1,000 rpm for 5 min.

The chromosome samples were produced by fixing (3×) with the Carnoy's solution (acetic acid:ethanol = 1:3) and abnormalities were counted after 5 min of staining with 5% Giemsa solution (Merck, NJ, USA, Lot No. HX888942). Two samples were made from each plate.

Two hundred metaphase cells (1,000 cells/plate) were observed per plate and classified according to structural abnormalities (gap of chromatid or chromosome;g, cutting of chromatid;ctb, exchange of chromatid;cte, cutting of chromosome; csb, exchange of chromosome; cse and others) and numerical abnormalities (pol).

Statistical analysis of the results was not performed. Results were evaluated as "positive" only when the percentage of chromosomal aberrations was >10%.

However the retest was performed in case that it was not confirmed the dosage dependency and the ratio of chromosomal aberration was unusually high in control solvent.

3. Results and discussion

3.1. Test for suppression of cell proliferation

The ratios of cell proliferation for the dosages of

MTBE were 86.7% and 84.4% at 1.25 mM and 2.5 mM respectively, for a 24 hour treatment using the direct method. Moreover cell proliferation ratios were 89.3%, 92.9%, 89.3%, 87.5% and 83.9% at 0.625 mM, 1.25 mM, 2.5 mM, 5 mM and 10 mM respectively for a 48 hour treatment. For the direct method, it was conformed to the good laboratory practice (GLP) guideline[22] that the maximum concentration is over 5 mg/mL in case of the cellular toxicity not being recognized. Cell proliferation ratios were 89.4%, 80.9% and 74.5% at 2.5 mM, 5 mM and 10 mM for a 6 hour treatment using the metabolic activated method.

The FA was 87.2%, 85.1% and 80.9% at 2.5 mM, 5 mM and 10 mM respectively for a 24 hour treatment using the direct method. Moreover cell proliferation ratios were 89.1%, 84.8% and 76.1% at 2.5 mM, 5 mM and 10 mM respectively for a 48 hour treatment. Cell proliferation ratios were 92%, 80% and 76% at 2.5 mM, 5mM and 10 mM for a 6 hour treatment using the metabolic activated method.

3.2. Chromosomal aberration test

Duplicate samples of 100 cells per plate were observed in metaphase and classified for structural abnormalities (gap of chromatid or chromosome;g, cutting of chromatid; ctb, exchange of chromatid; cte, cutting of chromosome;csb, exchange of chromosome; cse, etc) and numerical abnormalities (pol). Results were evaluated as being positive only when the percentage of chromosomal aberrations was 10% (20 abnormalities in 200 cells observed). A statistical analysis of the results was not performed.

Table 2. Chromosomal aberration test (direct method, 24 and 48 hr treatment) with MTBE

Treatment	Time of treatment (hr)	Conc. Of treatment (mM)	Obs. Cell No.	No. of diploid	No. of chromosomal structure abnormality								Decision
					gap	Chromatid		Chromosome		Etc.	Total		
					g	ctb	cte	csb	cse		-g	+g	
Control solvent (DMSO)	24	0	200	0	0.5	0	0	0	0	0	0	0.5	–
Test material	24	0.15625	200	0	0.5	0	0	0	0	0	0	0.5	-
		0.3125	200	0	0	0	0	0	0	0	0	0	–
		0.625	200	0	0	0	0	0	0	0	0	0	–
		1.25	200	0	1.5	0	0	0	0	0	0	1.5	–
		2.5	200	0	1	0	0	0	0	0	0	1	–
		5	200	0	1	0	0.5	0	0	0	0.5	1.5	–
		10	200	0	3	0	0	0	0	0	0	3	–
Positive control (MMC)	24	0.0004 (mg/mL)	200	0	11.5	6.5	37	0.5	0.5	0.5	45	56.5	+
Control solvent (DMSO)	48	0	200	0	0	0	0	0	0	0	0	0	–
Test material	48	0.15625	200	0	0	0	0	0	0	0	0	0	–
		0.3125	200	0	0.5	0	0	0	0	0	0	0.5	–
		0.625	200	0	0.5	0	0	0	0	0	0	0.5	–
		1.25	200	0	0.5	0	0	0	0	0	0	0.5	–
		2.5	200	0	0.5	0	0	0	0	0	0	0.5	–
		5	200	0	1	0	0.5	0	0	0	0.5	1.5	–
		10	200	0	1.5	0	1	0	0	0	1	2.5	–
Positive control (MMC)	48	0.0004 (mg/mL)	200	0	15.5	20.5	28.5	0	0	0	49	64.5	+

Conc.: concentration, Obs.: observed, No.: number, g: gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, MMC: mitomycin C.
-: negative, +: positive, -g: without gap, +g: with gap.

Table 3. Chromosomal aberration test (direct method, 24 and 48 hr treatment) with FA

Treatment	Time of treatment (hr)	Conc. Of treatment (mM)	Obs. Cell No.	No. of diploid	No. of chromosomal structure abnormality								Decision
					gap	Chromatid		Chromosome		Etc.	Total		
					g	ctb	cte	csb	cse		-g	+g	
Control solvent (DW)	24	0	200	0	1	0	0	0	0	0	0	1	−
Test material	24	0.15625	200	0	1	0	0	0	0	0	0	1	−
		0.3125	200	0	0	0	0	0	0	0	0	0	−
		0.625	200	0	1	0	0	0	0	0	0	1	−
		1.25	200	0	1	0.5	0	0	0	0	0.5	1.5	−
		2.5	200	0	0.5	0	0	0	0	0	0	0.5	−
		5	200	0	1	0	0	0	0	0	0	1	−
		10	200	0	2	0	0	0	0.5	0	0.5	2.5	−
Positive control (MMC)	24	0.0004 (mg/mL)	200	0	11	6.5	46	0	0.5	0.5	53.5	64.5	+
Control solvent (DW)	48	0	200	0	0.5	0	0	0	0	0	0	0.5	−
Test material	48	0.15625	200	0	0	0	0	0	0	0	0	0	−
		0.3125	200	0	0	0	0	0	0	0	0	0	−
		0.625	200	0	1	0	0	0	0	0	0	1	−
		1.25	200	0	0	0	0	0	0	0	0	0	−
		2.5	200	0	1	0	0	0	0	0	0	1	−
		5	200	0	1	0.5	0.5	0	0	0	1	2	−
		10	200	0	1	0	0	0	0	0	0	1	−
Positive control (MMC)	48	0.0004 (mg/mL)	200	0	16.5	20.5	28.5	0	0	0	49	65.5	+

Conc.: concentration, Obs.: observed, No.: number, g: gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, MMC: mitomycin C.
-: negative, +: positive, -g: without gap, +g: with gap.

The ratios of chromosomal aberration using the direct method are shown in Tables 2 and 3. No diploid presence was observed at any concentration after 24 and 48 hour treatment. The structural chromosomal aberrations were < 5.0% in both without gap (-gap) and with gap (+gap) groups. No dependency between chromosomal aberrations and dosages was observed.

The ratios of chromosomal aberration using the metabolic activated method are shown in Table 4. All results were the same as from the direct method. It was shown that the two chemicals do not induce any chromosomal aberrations, using either the direct method (24 hour and 48 hour treatment or the metabolic activated method, (6 hour treatment) in CHL/IU cells.

MTBE is not genotoxic but has induced tumors in rodents primarily at high concentrations that also induce other adverse effects[7]. No human data is available regarding potential genotoxic effects of MTBE. Limited genetic studies in rats have shown that MTBE may damage DNA and cause unscheduled DNA synthesis[9].

MTBE in oil was administered by gavage, 4 days per week for 104 weeks at 250 and 1000 mg/kg in a lifetime study in rats. An increase in Leydig interstitial cell tumors in males and a dose-related increase in leukemia and lymphoma in females was reported[24].

These results have been questioned because neither leukemias nor lymphomas were individually increased in treated females and because Leydig cell tumors are common in rats and do not predict the response to drugs or chemicals in humans[25]. As it is generally not

Table 4. Chromosomal aberration test (metabolic activated method, 6 hr treatment) with MTBE and FA

Treatment	Time of treatment (hr)	Conc. Of treatment (mM)	Obs. Cell No.	No. of diploid	No. of chromosomal structure abnormality								Decision
					gap	Chromatid		Chromosome		Etc.	Total		
					g	ctb	cte	csb	cse		-g	+g	
Control solvent (DMSO)	24 (6+18)	0	200	0	0.5	0	0	0	0	0	0	0.5	−
Test material (MTBE)	24 (6+18)	0.15625	200	0	0	0	0	0	0	0	0	0	−
		0.3125	200	0	1	0	0.5	0	0	0	0.5	1.5	−
		0.625	200	0	2.5	0	0	0	0	0	0	2.5	−
		1.25	200	0	1	0	0	0	0			1	−
		2.5	200	0	1	0	0	0	0	0	0	1	−
		5	200	0	0.5	0	0	0	0	0	0	0.5	−
		10	200	0	0.5	0	0	0	0	0	0	0.5	−
Positive control (CPA)	24 (6+18)	0.01 (mg/mL)	200	0	6.5	5	43	0.5	0	0	48.5	55	+
Control solvent (DW)	24 (6+18)	0	200	0	0.5	0	0	0	0	0	0	0.5	−
Test material (furfuryl alcohol)	24 (6+18)	0.15625	200	0	0	0	0	0	0	0	0	0	−
		0.3125	200	0	0.5	0	0	0	0.5	0	0	0.5	−
		0.625	200	0	0	0	0	0	1.5	0	1.5	1.5	−
		1.25	200	0	1	0	0	0	0	0	0	1	−
		2.5	200	0	1	0	0	0	0	0	0	1	−
		5	200	0	1	0	0	0	0	0	0	1	−
		10	200	0	1.5	0	0	0	0	0	0	1.5	−
Positive control (CPA)	24 (6+18)	0.01 (mg/mL)	200	0	9.5	13.5	36.5		0	0	50	59.5	+

Conc.: concentration, Obs.: observed, No.: number, g: gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, CPA: cyclophosphamide.
-: negative, +: positive, -g: without gap, +g: with gap.

mutagenic, MTBE-induced neoplasms occur through non-genetic mechanisms requiring chronic exposure to toxic doses unlikely to be voluntarily tolerated by humans because of the intense odor and taste at air or water concentrations predicted to result in cellular proliferation[25].

Using *in vitro* murine cell systems, various authors have argued for[26] or against[27] an important role for the formaldehyde metabolite in the carcinogenicity of MTBE.

MTBE by inhalation at airborne concentrations of 400, 3000, and 8000 ppm for 18 months in mice and 24 months in rats (6 hours/day, 5 days/week) produced an increased incidence of hepatocellular adenomas in female mice only and an increase in renal tubular cell tumors in male rats. The latter occur in association with nephropathy, which is believed to be unique to the rat. An increased incidence of Leydig cell tumors was found relative to controls, but this appears to be due to an unusually low incidence in the control group relative to historical controls[28].

MTBE was found to affect cell growth and induce cell transformation in cultured rodent fibroblasts[29].

Inhaled MTBE at an airborne concentration of 8000 ppm did not exhibit promotional activity for diethylnitrosamine induced hepatic foci in female mice[30].

In an unscheduled DNA synthesis assay, a dose-response relationship with rat primary hepatocytes was observed, meaning that MTBE may damage normal DNA and cause unscheduled DNA synthesis[31].

In vitro testing has produced equivocal results. A dose-related positive response was seen in the presence of metabolic activation in the mouse lymphoma

assay, while two Ames tests were negative[32].

MTBE was non-mutagenic in the *Drosophila* sex-linked recessive lethal test, rat bone marrow cytogenetic test, mouse bone marrow micronucleus test, and the *in vivo* & *in vitro* murine hepatic unscheduled DNA synthesis test[33].

The mutagenicity of MTBE in cultured mouse lymphoma cells was determined to be due to its metabolite, formaldehyde, from the greatly reduced mutagenic activity of MTBE in the presence of excess formaldehyde dehydrogenase. The latter enzyme would deactivate any formaldehyde formed to nonmutagenic formic acid[26]. However, the absence of a formaldehyde-induced pattern of DNA adducts in the livers of CD-1 female mice indicates that the hepatocellular adenomas induced in this strain by MTBE may not be the result of formaldehyde production[27].

MTBE was largely inactive for inducing sister chromatid exchanges and chromosome aberrations. It was inactive for sex-linked recessive lethal mutations in Drosophila melanogaster. It did not induce chromosome aberrations in rats *in vivo*[8]. It was negative in the *Drosophila* sex-linked lethal, mouse bone marrow micronucleus, and mouse hepatocyte unscheduled DNA synthesis systems[33].

FA was not mutagenic in *Salmonella typhimurium* strain TA 98, TA 100, TA 1535, or TA 1537 with or without metabolic activation. It did induce sister chromatid exchanges in cultured Chinese hamster ovary cells, but not with metabolic activation. No induction of chromosomal aberrations was noted in cultured Chinese hamster ovary cells treated with furfuryl alcohol without metabolic activation, but equivocal results were obtained with metabolic activation[34]. There was no increase in the frequency of sister chromatid exchanges in human lymphocytes treated with FA, nor was there any evidence of genotoxicity in *Drosophila melanogaster* in assays for sex-linked recessive lethal mutations and sex chromosome loss[34].

Under the conditions of these 2 yr inhalation studies, there was some evidence of carcinogenic activity of FA in male F344/N rats based on increasing incidences of combined neoplasms of the nose. There was equivocal evidence of carcinogenic activity in female F344/N rats based on marginally increasing incidences of neoplasms of the nose and renal tubule. There was some evidence of carcinogenic activity of furfuryl alcohol in male B6C3F1 mice based on increasing incidences of renal tubule neoplasms. There was no evidence of carcinogenic activity of furfuryl alcohol in female B6C3F1 mice exposed to 2, 8 or 32 ppm[35].

The induction of SCEs was also investigated in workers occupationally exposed to these solvents that are commonly used in the manufacture of furoic resins. The results obtained from the *in vitro* treatments show that furfural increased the number of SCEs, while FA did not. In exposed workers, neither of these solvents increased the spontaneous frequency of SCEs per metaphase[20].

Although most experiments will give clearly positive or negative results, in rare cases the data set will preclude making a definite judgment about the activity of the test substance. Results may remain equivocal or questionable regardless of the number of times the experiment is repeated. Positive results from the *in vitro* chromosome aberration test indicate that the test substance induces structural chromosome aberrations in cultured mammalian somatic cells. Negative results indicate that, under the test conditions, the test substance does not induce chromosome aberrations in cultured mammalian somatic cells.

From the results of this study, it was suggested the further investigations to perform such as FLARE assay (Comet assay with repair enzyme as Fpg, Endo III), real-time RT-PCR and even toxicogenomics studies. Moreover it would be more useful as a biomarker for chemical risk assessment to perform these tests with many chemicals.

Toxicological information from these studies could be used to prepare or update the MSDS in many industries for prevention occupational disease possibly occurs with using them.

4. Conclusion

This study was conducted because insufficient infor-

mation was available about the potential hazards of MTBE and FA. We are performed *in vitro* chromosomal aberration study with direct method and metabolic activated method, and it is concluded that these two chemicals are not mutagenic with these test conditions.

Acknowledgements

This work was supported by the Korea Occupational Safety & Health Agency, Ministry of Employment and Labor, Republic of Korea, and a Grant-in-Aid for chemical hazard identification, 2011.

References

1. Lide DR. *CRC Handbook of Chemistry and Physics.* 86th ed. **2005**, 2005-2006, p. 3-266, CRC Press, Taylor & Francis, Florida, U.S.A.
2. Lewis RJ. *Hawley's Condensed Chemical Dictionary.* 12th ed. **1993**, p. 760, Van Nostrand Rheinhold Co., New York, U.S.A.
3. Kirk-Othmer. *Encyclopedia of Chemical Technology.* 3rd ed., **1982**, Volumes 1-26, John Wiley and Sons Inc., New Jersey, U.S.A.
4. Kottke RH. Kirk-Othmer Encyclopedia of Chemical Technology, Furan Derivatives, **2005**, John Wiley & Sons, Inc., New Jersey, U.S.A.
5. Lewis RJ. *Hawley's Condensed Chemical Dictionary.* 14th ed. **2001**, John Wiley & Sons, Inc., New Jersey, U.S.A.
6. 21 CFR 175.105. U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of March 3, **2006**: http://www.gpoaccess.gov/ecfr
7. Environmental Health Criteria 206, Methyl tertiary-Butyl Ether, pp. 1-9 by the International Program on Chemical Safety (IPCS) under the joint sponsorship of the United Nations Environment Program, **1998**, International Labor Organization and the World Health Organization. Switzerland.
8. American Conference of Governmental Industrial Hygienists. TLVs and BEIs, Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices, **2008**, p. 39, U.S.A.
9. International Agency for Research on Cancer. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. **1999**, Lyon, France.
10. NIOSH. National Occupational Exposure Survey (NOES). **1983**.
11. Gosselin RE, Smith RP, Hodge HC. Clinical Toxicology of Commercial Products. 5th ed. **1984**, p. II-175, Williams and Wilkins, Baltimore, U.S.A.
12. Clayton GD, Clayton FE. Patty's Industrial Hygiene and Toxicology: Volume 2A, 2B, 2C: Toxicology. 3rd ed. 1981, p. 4654, John Wiley & Sons, Inc., New Jersey, U.S.A.
13. International Labor Organization, Encyclopedia of Occupational Health and Safety. 4th ed. **1998**, Volumes 1-4, p. 82, Geneva, Switzerland.
14. Bingham E, Cohrssen B, Powell CH. Patty's Toxicology, Volumes 1-9 5th ed. **2001**, p. 6:515, John Wiley & Sons, Inc., New Jersey, U.S.A.
15. O'Neil MJ. The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals, 13th ed. **2001**, p. 764, Merck and Co., Inc., New Jersey, U.S.A.
16. Budavari S. *The Merck Index* - Encyclopedia of Chemicals, Drugs and Biologicals, **1989**, p. 673, Merck and Co., Inc., New Jersey, U.S.A.
17. European Chemicals Bureau; IUCLID Dataset, Furfuryl alcohol (98-00-0) (2000 CD-ROM edition). Available from, as of February 15, 2006: http://esis.jrc.ec.europa.eu/
18. Lewis RJ. *Sax's Dangerous Properties of Industrial Materials.* 11th ed. **2004**, p. 1834, Wiley-Interscience, John Wiley & Sons, Inc., New Jersey, U.S.A.
19. Bringmann G, Kuhn R. Z. *Wasser-Abwasser-Forsch*, **1977**, 10(5), 161-6. Available from, as of January 23, 2012: http://cfpub.epa.gov/ecotox/quick_query.htm
20. Gomez-Arroyo S, Souza V. *In vitro* and occupational induction of sister-chromatid exchanges in human lymphocytes with furfuryl alcohol and furfural.*Mutat Res*, **1985**, 156(3), 233-8.
21. NIOSH. NIOSH Pocket Guide to Chemical Hazards & Other Databases CD-ROM. Department of Health & Human Services, Centers for Disease Prevention & Control. **2005**, National Institute for Occupational Safety & Health. DHHS Publication No. 2005-151.
22. Organization for Economic Co-operation and Development. Test No. 473: *In vitro* mammalian chromosome aberration test. OECD Guidelines for the Testing of Chemicals, **1997**, 1, 1-10.
23. Ishidate M Jr, Sofuni T. The *in vitro* chromosomal aberration test using Chinese Hamster Lung (CHL) fibroblast cells in culture. In: Ashby J, editor. *Progress in mutation research.* **1985**, 5, 427-32.
24. Belpoggi F, Soffritti M, Maltoni C. methyl tertiary butyl ether (MTBE) - a gasoline additive -- causes testicular and lymphohaematopoietic cancers in rats.

Toxicol Ind Health, **1995,** 11,119-149.
25. Mennear JH. Carcinogenicity Studies on MTBE: Critical Review and Interpretation. *Risk Analysis,* **1997,** 17(6), 673-681.
26. MacKerer CR, Angelo-Santo FA, Blackburn GR. Identification of formaldehyde as the metabolite responsible for the mutagenicity of methyl tertiary-butyl ether in the activated mouse lymphoma assay. *Proc Soc Exp Biol Med,* **1996,** 212, 338-341.
27. Casanova M, Heck HA. Lack of evidence for the involvement of formaldehyde in the hepatocarcinogenicity of methyl tertiary-butyl ether in CD-1 mice. *Chem Biol Interact,* **1997,** 105,131-143.
28. Bird MG, Burleigh-Flayer HD, Chun JS. Oncogenicity studies of inhaled methyl tertiary-butyl ether (MTBE) in CD-1 mice and F-344 rats. *J Appl Toxicol,* **1997,** 17, S45-S55.
29. Iavicoli I, Carelli G, Ardito R, Cittadini A, Sgambato A. Methyl *tertiary*-butyl ether (MTBE) inhibits growth and induces cell transformation in rodent fibroblasts *Anticancer Res,* **2002,** 22, 2173-2178.
30. Moser GJ, Wong BA, Wolf DC. Methyl tertiary butyl ether lacks tumor-promoting activity in N-nitrosodiethylamine-initiated B6C3F1 female mouse liver. *Carcinogenesis,* **1996,** 17, 2753-2761.
31. Zhou W, Yuan D, Huang G. Mutagenicity of methyl tertiary butyl ether. *J Environ Pathol Toxical Oncol,* **2000,** 19, 35-39.
32. Neal MW, Anatra M, Jacobson RA. Technical Support Document, Methyl tert-Butyl Ether, **1987,** Environmental Protection Agency, Washington DC, U.S.A.
33. McKee RH, Vergnes JS, Galvin JB. Assessment of the *in vivo* mutagenic potential of methyl tertiary-butyl ether. *J Appl Toxicol,* **1997,** 17, S31-S36.
34. Bingham E, Cohrssen B, Powell CH. *Patty's Toxicology.* Volumes 1-9 5th ed. **2001,** p. 6:515, John Wiley & Sons. New York, U,S,A,
35. National Institute of Health. Toxicology & Carcinogenesis Studies of Furfuryl Alcohol in F344/N Rats and B6C3F1 Mice, Technical Report Series No. 482. **1999,** Publication No. 99-3972 U.S. Department of Health and Human Services, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, U.S.A.