Chromosome aberrations in bone marrow cells of rats treated with MTBE

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Abstract: In the present study, genotoxic effect of methyl tert butyl ether MTBE was analyzed by measuring chromosomal aberrations (CAs) in bone marrow cells of rats. Rats administered MTBE orally at 800, 1600mg/kg/day in corn oil for 14 and 28 consecutive days. Control rats received injection of distilled water. An additional two groups of rats received corn oil and served as vehicle controls. Treatment of corn oil for 14 and 28 days failed to induce chromosomal aberrations. The highest percentage of chromosomal aberrations was produced by the two tested dose 14 days after treatment. The most structural aberrations were Robertsonion translocations, deletion, dicentric, end to end association while, ring, acentric fragment and gaps were rare. The present results indicate that MTBE is harmful to mammalian genetic material.

Keywords: Methyl tert butyl ether (MTBE), chromosomal aberrations assay (CAA), male rats *Rattus norvegicus*.

INTRODUCTION

Methyl tert-butyl ether (MTBE) is a well-known environmental contaminant owing to its high solubility in water (Sawunyama and Bailey, 2002). MTBE is widely used as an additive to gasoline, to increase oxygen content and reduce tailpipe emission of carbon monoxide (Perbellini *et al.,* 2003). Since MTBE is resistant to most physical methods of treating fuel-contaminated water, biodegradation may be a useful means of remediation (Youngster *et al.,* 2008). MTBE had potential uses as an anti-angiogenic treatment for solid tumors with minimal toxicity and constitutes a good solvent for mixed cholesterol stones (Kozlosky *et al.,* 2013 and Dai *et al.*, 1988). MTBE-induced vascular toxicity in zebrafish (*Danio rerio*), embryos (Bonventre *et al.,* 2011); induced cell injury, associated with mitochondrial dysfunction, and alterations in cytosolic Ca^{2+} in isolated rabbit tracheal epithelial cells (Wang *et al.,* 2008); resulted in mild changes in hormone levels and endocrine-sensitive tissues in rats (Williams *et al.*, 2000) and caused an increased incidence of Leydig cell testicular tumors in male rats, a dose-related increase of leukemias, an increase of dysplastic proliferations of lymphoreticular tissues and also an increase of uterine sarcomas in females (Belpoggi *et al.,* 1997).

However, Werner *et al.* (2001) suggested that at environmental MTBE exposure levels found in surface waters (<0.1 mg/l), this compound was likely not acutely toxic to aquatic life.

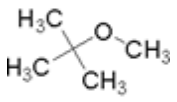
Moreover, MTBE could induce DNA double-strand breaks and inhibit cell growth *in vitro* (Song *et al.,* 2002 and Chen *et al.,* 2008). Also, the mutagenic effects of methyl tertiary-butyl ether (MTBE) in *Salmonella typhimurium* TA102, in the presence of an exogenous metabolic activation system could not be confirmed (McGregor *et al.,* 2005).

The present study aimed to investigate the chromosomal aberrations induced in rat's bone marrow cells under the effect of MTBE.

MATERIALS AND METHODS

Chemical

Methyl tert-Butyl ether (MTBE) was obtained from Sigma Chemical Company. MTBE Cas Number: 1634-04-4; Formula: $C_5H_{12}O$; Molecular weight: 88.15; melting point: -109°C; boiling point: 55.2°C; density: 0.74, Refractive Index: 1.3689 and the purity was more than 99% (GC≥99.9%). The compound is highly volatile (US EPA, 1997). The structural formula is showed in fig. 1 as stated by Belpoggi *et al.* (1995).



Animals

Adult male rats aged (50-100) weeks and weighing 50-70g were employed. The animals were maintained under proper environmental conditions, i.e. temperature 25±2°C, humidity 50±5% with a 12h light/dark period. They were housed in cages and fed with pelleted standard diet and tap water ad libitum. The animals were allowed to acclimatize for at least 5 days prior to the study. Six mice were used for each dose and each sampling time. All experiments were conducted according to the protocol

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approved by Faculty of Medicine Ethics Committee (MFEC), Ain Shams University, Egypt.

Treatment

The rats were divided into seven groups treated via oral gavage. The first group (control) treated with distilled water. Groups 2, 3 & 4 received corn oil, 800mg/kg b.w. and1600mg/kg b. w. for 14 days, respectively. Groups 5, 6 & 7 received corn oil, 800mg/kg b.w. and 1600mg/kg b. w. for 28 days, respectively. These doses were chosen according to Li *et al.* (2008). The vehicle was corn oil. Dilution of MTBE in corn oil was made before the start of the test and each day prior to test renewal in a 5ml volumetric flask. Rats were tested to ensure that there was no evidence of infectious pathogens.

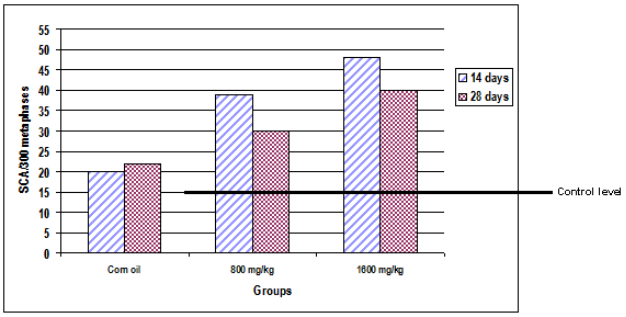

Fig. 1: Structural chromosomal aberrations in bone marrow cells induced by MTBE via the oral gavage route

Chromosome aberration studies

For chromosomal aberrations, rats at all fixation intervals were injected intraperitoneally with 0.04% colchicine solution, 0.5 ml/10g body weight 2h prior to sacrifice. Bone marrow from the femur was flushed out into 0.56% KCl hypotonic solution. Cells were centrifuged and fixed with two or three changes of Carnoy's fixative (3: 1 methanol- acetic acid). The slides were prepared by flame-drying and stained with buffered Giemsa (pH 6.8). Fifty metaphase spreads per animal were analyzed for chromosomal aberrations (Tice *et al.*, 1994).

STATISTICAL ANALYSIS

Statistical significant was evaluated using an ANOVA (one-way) test following by Tukey test. Statistical significance was accepted if $p \leq 0.05$.

RESULTS

Chromosome aberrations assay: Among control group, the total chromosomal aberrations were spotted on 5±3.28% metaphases from 300 metaphases. The frequencies of chromosome aberrations were not significantly increased in rats treated with corn oil for 14 or 28 days (table 1). The highest percentage of aberrations was produced by the two tested dose 14 days after treatment. A higher level of chromosomal aberrations was found at the highest concentration of MTBE for 14 days. The percentage structural chromosomal aberrations for rats treated with 1600 mg MTBE decreased from 16±6.06% after 14 days to 13.33±7.11% after 28 days. The chromosomal aberrations percentages in rats was reached 13±5.17 after oral administration of MTBE at 800 mg for 14 consecutive days. This percentage decreased to 10±6.19 in bone-marrow cells after 28 days and only 1.66% of them had chromatid deletions (table 1). The most structural aberrations were Robertsonian translocation, dicentric and deletions, while ring, gap and acentric fragments were similar in number. Other aberrations such as breaks were not spotted. Deleted chromosomes occurred in 2.33% of the examined metaphases 14 days post-treatment with the small dose. Chromosomal exchanges were also observed at certain instances. Acentric fragments occurred as small parts of chromosomes, each having no apparent centromere. Fragments were single or double, fig. (2). It is of interest to state that, some metaphase spreads might have more than one type of structural aberrations. Cells with more than one aberrations were grouped as multiple aberrations.

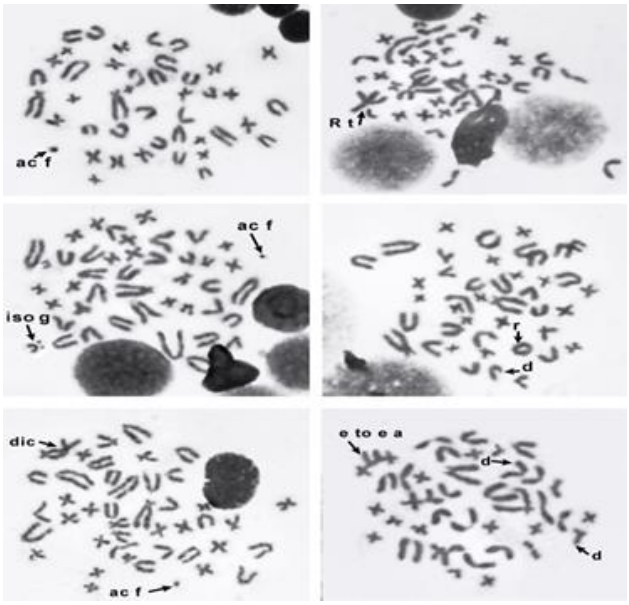

Fig. 2: Structural chromosomal aberrations in bone marrow cells induced by MTBE via the oral gavage rout.
R t= Robertsonian translocation, e to e a= end to end association, d= deletion,
dic= dicentric, ac f= acentric fragment, r= ring, g= gap

DISCUSSION

Rats were assessed at two intervals, against suitable controls, using parameters like chromosomal aberrations. Because a previous study demonstrated a decrease in plasma testosterone in male Sprague-Dawley rats treated orally with 800 mg MTBE /kg/day for 28 days (Day *et al.*, 1998), a 28-day MTBE treatment was used along with

Table 1: The percentage of the different types of structural chromosomal aberrations in male rats treated with methyl tert butyl ether

Experimental group	Duration	Number of examined metaphases	Structural chromosomal aberrations/300 cells							Total	Mean ±S.D%
			R.t.	e to e ass	del	dic	ac.f.	r.	g		
control	0	300	2	1	1	6	2	1	2	15	5.±3.28
Corn oil	14 days	300	3	1	2	5	4	4	1	20	6.66±1.63^{-}
800mg/kg b.w.		300	12	6	7	7	3	4	-	39	13±5.17^{-}
1600mg/kg b.w.		300	12	2	9	11	4	6	4	48	16±6.06*,**
Corn oil	28 days	300	3	4	5	3	1	4	2	22	7.33±3.26^{-}
800mg/kg b.w.		300	5	10	5	4	1	-	5	30	10±6.19^{-}
1600mg/kg b.w.		300	7	5	9	6	5	4	4	40	13.33±7.11^{-}

SCA=Structural chromosomal aberrations, R t = Robertsonian translocations, e to e a= end to end association, d= deletion, dic= dicentric, ac f =acentric fragment, r= ring, g = gap, SCA=Structural chromosomal aberrations, - = non significant $p \geq 0.05$, *$p \leq 0.05$ versus corn oil group, **$p \leq 0.01$ versus control group

a 15-day treatment to compare possible length of exposure differences. They indicated that MTBE alters endocrine-sensitive parameters in adult male rats.

MTBE clearly induced chromosome aberrations under the treatment conditions. It has also been shown in the present study that chromosomal aberrations (CAs) induction increases in dose-dependent manner. The present observations were agreements with the results obtained by Li *et al.* (2008). They suggested that relatively high dose of MTBE could be exerted reproductive system toxicity of male rats and disturbed the secretion of testosterone, luteinizing hormone and follicle stimulating hormone, possibly due to oxidative stress induced by treatment of MTBE for 2 weeks.

Corn oil alone did not induce chromosomal damage in bone marrow cells. The induction of chromosome aberrations was significantly increased in rats after multiple oral treatments of 1600 mg MTBE Kg^{-1}b.w. The chromosomal aberrations percentage occurred at a higher rate after 14 days of 1600mg MTBE /kg b.w. The results demonstrated that the number of abnormal metaphases identified in the 14 day treatments is higher than that observed in the 28 day treatment, for the two treated group. As suggested by Gautam and Kapoor (1991) such reduction may be due to the fact that cells with severe chromosomal damage might have been deleted in cell cycle following the treatment. Then the level of chromosomal aberrations decreased after 28 days of MTBE treatment, although still higher than the control level. The observed cytological aberrations in the bone marrow assay reveal that MTBE has the potentiality to induce genotoxicity at the chromosome level.

To the author′s opinion, the drug causes chromosome aberration which seems to be reversible as the drug eliminated or diminished from the bone marrow through the metabolic process. Such level of chromosome aberrations might be related to the usual decrease of DNA adduct in rats. The elimination half-life (t1/2) of MTBE was approximately the same after single low-and high-concentration exposures (0.52 and 0.63 hours, respectively). After the repeat exposures, the MTBE t1/2 was slightly shorter 0.48 and 0.51 hours, respectively (Integrated Risk Information System, IRIS 1991). Between 35 and 69% of the MTBE retained after the end of the exposure was recovered as metabolites in urine of both humans and rats (Amberg *et al.*, 1999).

Moreover, it is possible that MTBE could also express an aneugenic mode of action as inhibiting cell division and mitotic spindle apparatus. The chromosomal aberrations observed in animals clearly indicate that this compound interacts with chromatin DNA and induce damage there (data not published).

Statistically significant chromosomal aberrations, micronucleus and sperm abnormalities revealed the genotoxicity of a test compound as stated by Jayashree *et al.* (1994). Similarly, chromosomal damage is considered to detect early effects of xenobiotic insults and elevation of the frequency of CAs is a sensitive cytogenetic assay for detecting exposure to mutagens and carcinogens (Bonassi *et al.*, 1995; Sierra-Tores *et al.*, 1998).

Robertsonian translocations, deletions and dicentric were increased at two doses studied indicating the clastogenic potential of MTBE. These findings suggest that G1 could be the cell cycle phase most sensitive to MTBE genetic damage. MTBE could induce the higher expression of c-mycprotooncogene, which suggested it could promote cell proliferation-one of possible mechanisms of carcinogenesis in animals (Zhou *et al.*, 1999).

Giannotti *et al.* (2002) mentioned that strand breaks are too short lived to allow detection after a 3h treatment period (due to preferential repair), indicating the need for shorter exposure times in some cases to optimize their detection.

Chromosomal aberrations qualitatively and quantitatively detect clastogenic activity, while the micronucleus assay detects both clastogenic effects and damage to the mitotic apparatus, some of which might have aneugenic consequences (Dimitrov *et al.*, 2006).

Moreover, several authors reported the genotoxic effects of MTBE *in vivo* and/or *in vitro*. Accordingly, Williams-Hill *et al.* (1999) concluded that MTBE and its metabolites induce a mutagenic pathway involving oxidation of DNA bases and an intact repair system.

Yuan *et al.* (2007) concluded that the methyl group of MTBE and tert-butyl alcohol definitely form adducts with DNA in mouse liver, lung and kidney. The methyl group of MTBE is the predominant binding part in liver, while the methyl group and the tert-butyl groups give comparable contributions to the adduct formation in lung and kidney. Bonventre *et al.* (2011) observed significant decrease in the expression of vegfa, vegfc and flk1/kdr in vascular development following embryonic exposure to MTBE.

Also, Ghasemi and Ahmadi (2014) revealed that MTBE may have interaction with calf thymus DNA (ct-DNA) via the minor groove of DNA. Also, MTBE may be complexed into the basket of G-quadruplex structure.

Recently, Bravo *et al.* (2015) reported that the expression of alkane monooxygenase (alkB) gene was related to the co-metabolic oxidation of MTBE. Valipour *et al.* (2015) showed that insulin formed a molten globule (MG)-like structure in the presence of 8uM MTBE due to protein oxidation and reactive oxygen specious (ROS) generation.

CONCLUSION

The present results demonstrated that MTBE has a clastogenic potential as measured by the bone marrow chromosomal aberrations in rats.

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REFERENCES

Amberg A, Rosner E and Dekant W (1999). Biotransformation and kinetics of excretion of methyl-tert-butyl ether in rats and humans. *Toxicol. Sci.*, **51**(1): 1-8.

Belpoggi F, Soffritti M and Malton C (1995). Methyl-tertiary-butyl ether (MTBE)-a gasoline additive-causes testicular and lympho-haematopoietic cancers in rat. *Toxicol. Industr. Health*, **11**(2): 119-149.

Belpoggi F, Soffritti M, Filippini F and Maltoni C (1997). Results of long-term experimental studies on the carcinogenicity of methyl tert-butyl ether. *Ann. N. Y. Acad. Sci.*, **837**: 77-95.

Bonassi S, Abbondandolo A and Camurri L *et al*. (1995). Are chromosome aberrations in circulating lymphocytes predictive of future cancer onset in humans? Preliminary results of an Italian cohort study. *Cancer Genetics and Cytogenetics,* **79**(2): 133-135.

Bonventre JA, White LA and Cooper KR (2011). Methyl tert butyl ether targets developing vasculature in zebrafish (*Danio rerio*) embryos. *Aquat. Toxicol.*, **105**(0): 29-40.

Bravo AL, Sigala JC, Le Borgne S and Morales M (2015). Expression of an alkane monooxygenase (alkB) gene and methyl tert-butyl ether co-metabolic oxidation in *Pseudomonas citronellolis*. *Biotechnol. Lett.*, **37**(4): 807-814.

Chen CS, Hseu YC, Liang SH, Kuo JY and Chen SC (2008). Assessment of genotoxicity of methyl-tert-butyl ether, benzene, toluene, ethylbenzene and xylene to human lymphocytes using comet assay. *Journal of Hazardous Materials*, **153**(1-2): 351-356.

Dai KY, Montet JC, Zhao XM, Amic J and Choux R (1988). Dissolving agents of human mixed cholesterol stones. *Gastroenterol. Clin. Biol.*, **12**(4): 312-319.

Day KJ, de Peyster A, Allgaier BS, Luong A and MacGregor JA (1998). Methyl t-butyl ether (MTBE) effects on the male rat reproductive endocrine axis. *Toxicol.*, **42**: 174.

Dimitrov BD, Gadeva PG, Benova DK and Bineva MV (2006). Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems. *Mutagenesis*, **21**(6): 375-382.

Gautam DC and Kapoor L (1991). Genotoxic effects of dithane-M-45 on the bone marrow cells of mice *in vivo*. *Experientia*, **47**: 280-282.

Ghasemi S and Ahmadi F (2014). The study of binding of methyl tert-butyl ether to human telomeric G-quadruplex and calf thymus DNA by gas chromatography, a thermodynamic discussion. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, **971**: 112-119.

Giannotti E, Vandin L, Repeto P and Comelli R (2002). A comparison of the *in vitro* Comet assay with the *in vitro* chromosome aberration assay using whole human blood or Chinese hamster lung cells: Validation study using a range of novel pharmaceuticals. *Mutagenesis*, **17**(2): 163-170.

Integrated Risk Information System (IRIS) (1991). US Environmental Protection Agency. National Center for Environmental Assessment. Methyl tert-butyl ether (MTBE); CASRN 1634-04-4.

Jayashree IV, Vijayalaxmi KK and Abdul Rahiman M (1994). The genotoxicity of Hinosan, an organophosphorus pesticide in the *in vivo* mouse. *Mutat. Res.*, **322**(2): 77-85.

Kozlosky J, Bonventre J and Cooper K (2013). Methyl tert butyl ether is anti-angiogenic in both *in vitro* and *in vivo* mammalian model systems. *J. Appl. Toxicol.*, **33**(8): 820-827.

Li D, Yuan C, Gong Y, Huang Y and Han X (2008).The effects of methyl tert-butyl ether (MTBE) on the male rat reproductive system. *Food Chem. Toxicol.,* **46**(7): 2402-2408.

McGregor DB, Cruzan G, Callander RD, May K and Banton M (2005). The mutagenicity testing of tertiary-butyl alcohol, tertiary-butyl acetate and methyl tertiary-butyl ether in *Salmonella typhimurium*. *Mutat. Res.*, **565**: 181-189.

Perbellini L, Pasini F, Prigioni P and Rosina A (2003). Occupational exposure to methyl tert-butyl ether (MTBE) at an oil refinery. *G. Ital. Med. Lav. Ergon.*, **25**(3): 39-40.

Sawunyama P and Bailey GW (2002). Methyl tert-butylether-water interaction. Presented at American Society of Agronomy-Crop Science Society of America-Soil Science Society of America Annual Meeting, Indianapolis, IN, November 10-14.

Sierra-Tores CH, Cajas-Salazar N, Hoyos LS and Zuleta M *et al.* (1998). *In vitro* and *in vivo* genotoxic activity of miral, an organophosphorus insecticide used in Colombia. *Mutat. Res.*, **415**: 59-67.

Song C, Zhang Z, Chen X, Zhang Y, Wang C and Liu K (2002). Study on three kinds of gasoline oxygenates-induced DNA damage in mice fibroblasts. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi.,* **20**(5): 362-364.

Tice RR, Hayashi M, MacGregor JT, Anderson D, Blakey DH, Holden HE, Kirsch-Volders M, Oleson FB Jr, Pacchierotti F and Preston RJ *et al.* (1994). Report from the working group on the *in vivo* mammalian bone marrow chromosomal aberration test. *Mutat. Res.*, **312**: 305-312.

US EPA (1997). Drinking Water Advisory: Consumer Acceptability Advice and Health Effects Analysis on Methyl Tertiary-butyl Ether (MTBE)(EPA-822-F-97-009). US Environmental Protection Agency, Office of Water, Washington, DC, USA.

Valipour, M, Maghami, P, Habibi-Rezaei M, Sadeghpour M, Khademian MA, Mosavi K, Sheibani N and Moosavi-Movahedi AA (2015). Interaction of insulin with methyl tert-butyl ether promotes molten globule-like state and production of reactive oxygen species. *Int. J. Biol. Macromol.*, **80**: 610-614.

Wang Y, Chen C, Wu T, Xu J and Han X (2008). Methyl tert-butyl ether (MTBE) induced Ca^{2+}-dependent cytotoxicity in isolated rabbit tracheal epithelial cells. *Toxicol. In vitro*, **22**(7): 1734-1741.

Werner I, Koger CS, Deanovic LA and Hinton DE (2001). Toxicity of methyl-tert-butyl ether to freshwater organisms. *Environ. Pollut.*, **111**(1): 83-88.

Williams-Hill D, Spears CP, Prakash S, Olah GA, Shamma T, Moin T, Kim LY and Hill CK (1999). Mutagenicity studies of methyl-tert-butyl ether using the Ames tester strain TA102. *Mutat. Res.*, **446**(1): 15-21.

Williams TM, Cattley RC and Borghoff SJ (2000). Alterations in endocrine responses in male Sprague-Dawley rats following oral administration of methyl tert-butyl ether. *Toxicol. Sci.,* **54**: 168-176.

Youngster LK, Somsamak P and Häggblom MM (2008). Effects of co-substrates and inhibitors on the anaerobic O-demethylation of methyl tert-butyl ether (MTBE). *Appl. Microbiol. Biotechnol.,* **80**(6): 1113-1120.

Yuan Y, Wang HF, Sun HF, Du HF, Xu LH, Liu YF, Ding XF, Fu DP and Liu KX (2007). Adduction of DNA with MTBE and TBA in mice studied by accelerator mass spectrometry. *Environ. Toxicol.,* **22**(6): 630-635.

Zhou W, Ye S and Zhu Y (1999). Effects of methyl tertiary butyl ether on liver tissue gene expression in rats. *Zhonghua Yu Fang Yi Xue Za Zhi*., **33**(2): 85-87.