METHYL-TERTIARY-BUTYL ETHER (MTBE) — A GASOLINE ADDITIVE — CAUSES TESTICULAR AND LYMPHO-HAEMATOPOIETIC CANCERS IN RATS

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In the framework of a series of experiments conducted to evaluate the carcinogenic effects of oxygenated gasoline additives, MTBE was analyzed in an oral lifetime carcinogenicity study using 8-week-old male and female Sprague-Dawley rats. These experiments were part of a large research project on gasoline carcinogenicity performed at the Bentivoglio (BT) Castle Cancer Research Center of the Ramazzini Foundation and of the Bologna Institute of Oncology. MTBE, dissolved in oil, was administered by stomach tube at the doses of 1000, 250, or 0 mg/kg b.w., once daily, four days weekly, for 104 weeks. The animals were maintained until natural death. The last animal died 166 weeks after the start of the experiment, i.e., at 174 weeks of age. Under the tested experimental conditions, MTBE was shown to cause an increase in Leydig interstitial cell tumors of the testes and a dose-related increase in lymphomas and leukemias in female rats.

INTRODUCTION

Methyl-tertiary-butyl ether (MTBE) is one of the most widely produced synthetic compounds in the world. Its major, if not unique use, is as an oxygenated additive to gasoline intended to improve the combustion process and more specifically, to significantly reduce motor vehicle CO emission, especially during low temperatures in winter months.

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2. Abbreviations: BT, Bentivoglio; DIPE, di-isopropyl ether; GLP, good laboratory practices; MON, motor octane number; ETBE, ethyl-tertiary-butyl ether; MTBE, methyl-tertiary-butyl ether; RON, research octane number; SOP, standard operating procedures; TAME, tert-amyl-methyl ether.

3. Key words: leukemia, Leydig cell testicular tumor, MTBE, rat.

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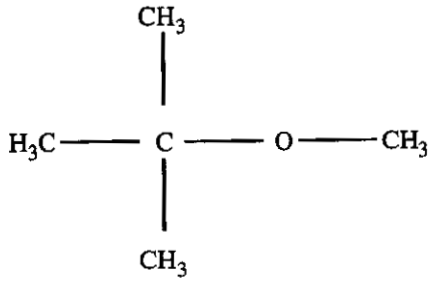
MTBE ($C_5H_{12}O$), which is produced by reacting isobutylene with methanol, is a clear, colorless liquid, with a characteristic terpenic odor. Its structural formula is:



The properties of the compound are shown on Table 1.

TABLE 1. Properties of MTBE [1]

Property	Values
Molecular weight	88
Specific gravity (15.6°C)	0.747
Net energy content, kJ/g	34.9
Reid vapor pressure, kPa	53.6
Boiling point, °C	55
Heat of vaporization, J/g	320
Octane rating[2]	
RON	117
MON	102
Water solubility at 21°C	
MTBE in water, vol. %	6.9
Water in MTBE, vol. %	1.4
Stoichiometric A/F	11.7

[1]From CHEMTECH, 1979.
[2]RON: research octane number; MON: motor octane number.

Presently, MTBE is produced worldwide at a rate of more than 16 million tons per year. It is mainly consumed in the United States, particularly in the cooler states. In these cooler states, it is added to gasoline at concentrations of up to 15%. During the period 1984–1988, the percent of MTBE-containing gasoline increased from 8 to 22% in these states. In 1995, MTBE use in the United States is estimated to average 283,900 b/d, equivalent to 12.3 million tons/year (Williams, 1995). In Italy, where the industrial production of MTBE began in 1985 and its commercial expansion as a gasoline optimizer began in 1992, 300 thousand tons are produced annually. Since MTBE is added to gasoline at such high concentrations, it must not

be considered as only an additive, but a true basic component of oxygenated gasolines. The use of MTBE as a component of gasoline has been recommended on the assumption that it would ameliorate the quality of the combustion exhausts, in terms of environmental and health safety (API, 1988; U.S. EPA, 1988).

Data on the long-term health effects of MTBE were not available at the time its use as an additive in premium gasoline was recommended and initiated in the 1970s. It is astonishing that such a technological process could have been started without sufficient toxicological information that would have enabled us to expose possible adverse health effects of the compound.

In 1988, the production and use of MTBE was already significant. Further, its use was predicted to expand, not only domestically but worldwide. We therefore began an early experimental research study (long-term bioassays) to assess if, and to what extent, MTBE could pose carcinogenic risks. This research was planned to provide qualitative, primary information. The study would be further investigated through larger, long-term carcinogenicity bioassays with the correlated mechanistic study.

This study was a part of a vast series of experimental research investigations performed at the Cancer Research Center of the Ramazzini Foundation and of the Bologna Institute of Oncology, at the Bentivoglio (BT) Castle, aimed at studying the carcinogenic risk of oxygenated gasoline additives presently used and proposed as gasoline optimizers. The plan and the state-of-the-art of this series of experiments are given in Table 2. All the experiments were performed on Sprague-Dawley rats, kept under observation for life span, following good laboratory practices (GLP) and applying the same standard operating procedures (SOP).

This report deals with the results of our experiments on the carcinogenicity of MTBE. Preliminary results were given at the Collegium Ramazzini Conference on "PRESENT KNOWLEDGE ON THE HAZARDS OF CONVENTIONAL AND NEW GASOLINES" held in Carpi, Italy, on October 29, 1993, in the framework of the "Annual Ramazzini Days, 1993."

MATERIALS AND METHODS

The purity of the MTBE, supplied by a domestic (United States) gasoline company, was higher than 99%. During the experiment, it was stored at a temperature of 4°C.

Male and female Sprague-Dawley rats of the colony at the BT Cancer Research Center were used. This colony of rats has been used for various experiments in the BT Laboratory for nearly 25 years. Historical data are available on more than 10,000 historical controls, kept under observation for lifespan and submitted to systematic necropsies and standardized histopathological examinations. Therefore, data on the expected incidence of the different types of tumors in control animals and its fluctuations are available.

TABLE 2. BT Plan of the Series of Long-Term (Life-Span) Carcinogenicity Bioassays of Oxygenated Gasoline *Additives*, and of Oxygenated Additive-Containing Gasolines, Performed on Sprague-Dawley Rats[1]

(Part I)

Compound	No. (and code) of the experiment	Experimental group	Treatment			Animals			State of the art
			Dose	Route	Type of administration	Sex	No.	Age at start (weeks)	
Methyl alcohol	1 (BT 7001)	I	15 mg/l	Ingestion	In drinking water supplied ad libitum for 104 weeks	M F	50 50	7	Histopathologically evaluated; *results in press*
		II	0 (control)			M F	100 100		
	2 (BT 960)	I	20,000 mg/l	Ingestion	In drinking water supplied ad libitum for 104 weeks	M F	100 100	8	Biophase concluded
		II	5,000 mg/l			M F	100 100		
		III	500 mg/l			M F	100 100		
		IV	0 (control)			M F	100 100		
	3 (BT 961)	I	24 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M F	60 60		*108 weeks of biophase*
		II	0[2] control			M F	60 60		

[1]Experiments 3, 5, 7, and 10 have the control group in common; experiments 8 and 9 have the control group in common.
[2]Olive oil alone.

TABLE 2. BT Plan of the Series of Long-Term (Life-Span) Carcinogenicity Bioassays of Oxygenated Gasoline Additives, and of Oxygenated Additive-Containing Gasolines, Performed on Sprague-Dawley Rats[1]

(Part II)

Compound	No. (and code) of the experiment	Experimental group	Treatment			Animals			State of the art
			Dose	Route	Type of administration	Sex	No.	Age at start (weeks)	
Ethyl alcohol	4 (BT 6004)	I	10% v/v	Ingestion	In drinking water administered ad libitum, lifespan	M F	110 110	39[3] (breeders)	Biophase concluded: histopathological evaluation is ongoing
		II	10% v/v			M F	30 39	Embryos[4] (offspring)	
		III	0 (control)			M F	110 110	39[3] (breeders)	
		IV	0 (control)			M F	49 55	Embryos[4] (offspring)	
	5 (BT 961)	I	40 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M F	60 60	8	108 weeks of biophase
		II	0[2] (control)			M F	60 60		

[1]Experiments 3, 5, 7, and 10 have the control group in common; experiments 8 and 9 have the control group in common.
[2]Olive oil alone.
[3]From 7 days before mating.
[4]From the conception.

TABLE 2. BT Plan of the Series of Long-Term (Life-Span) Carcinogenicity Bioassays of Oxygenated Gasoline Additives, and of Oxygenated Additive-Containing Gasolines, Performed on Sprague-Dawley Rats[1]

(Part III)

Compound	No. (and code) of the experiment	Experimental group	Treatment			Animals			State of the art
			Dose	Route	Type of administration	Sex	No.	Age at start (weeks)	
MTBE	6 (BT 958)	I	1,000 mg/kg b.w	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M F	60 60	8	Results verbally presented at the Ramazzini Days in Carpi, Italy, October 1993 – This report
		II	250 mg/kg b.w.			M F	60 60		
		III	0[2] (control)			M F	60 60		
ETBE	7 (BT 959)	I	1,000 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M F	60 60	8	108 weeks of biophase
		II	250 mg/kg b.w.			M F	60 60		
		III	0[2] (control)			M F	60 60		

[1]Experiments 3, 5, 7, and 10 have the control group in common; experiments 8 and 9 have the control group in common.
[2] Olive oil alone.

TABLE 2. BT Plan of the Series of Long-Term (Life-Span) Carcinogenicity Bioassays of Oxygenated Gasoline Additives, and of Oxygenated Additive-Containing Gasolines, Performed on Sprague-Dawley Rats[1]

(Part IV)

Compound	No. (and code) of the experiment	Experimental group	Treatment			Animals			State of the art
			Dose	Route	Type of administration	Sex	No.	Age at start (weeks)	
TAME	8 (BT 963)	I	750 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M F	100 100	8	12 weeks of biophase
		II	250 mg/kg b.w.			M F	100 100		
		III	0[2] (control)			M F	100 100		
DIPE	9 (BT 964)	I	1,000 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M F	100 100	8	12 weeks of biophase
		II	250 mg/kg b.w.			M F	100 100		
		III	0[2] (control)			M F	100 100		

[1]Experiments 3, 5, 7, and 10 have the control group in common; experiments 8 and 9 have the control group in common.
[2]Olive oil alone.

TABLE 2. BT Plan of the Series of Long-Term (Life-Span) Carcinogenicity Bioassays of Oxygenated Gasoline Additives, and of Oxygenated Additive-Containing Gasolines, Performed on Sprague-Dawley Rats[1]

(Part V)

Compound	No. (and code) of the experiment	Experimental group	Treatment			Animals			State of the art
			Dose	Route	Type of administration	Sex	No.	Age at start (weeks)	
Gasoline containing 3% methyl alcohol	10 (BT 961)	I	800 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M F	60 60	8	108 weeks of biophase
Gasoline containing 5% ethyl alcohol		II	800 mg/kg b.w.			M F	60 60		
Gasoline		III	768 mg/kg b.w.			M F	60 60		
		IV	0[2] control			M F	60 60		
Gasoline containing 15% MTBE	11 (BT 962)	I	800 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M F	60 60	8	75 weeks of biophase
Gasoline containing 15% ETBE		II	800 mg/kg b.w.			M F	60 60		
		III	0[2] (control)			M F	60 60		

[1]Experiments 3, 5, 7, and 10 have the control group in common; experiments 8 and 9 have the control group in common.
[2]Olive oil alone.

After weaning, at 4–5 weeks of age, the experimental animals were identified by ear punch, randomized in order to have no more than one male and one female of each litter in the same group, and housed five per cage. The animals were eight weeks old at the start of the experiment.

The plan of the experiment is shown in Table 3.

TABLE 3. Long-Term Carcinogenicity Bioassays on Methyl-Tertiary-Butyl Ether (MTBE), Administered by Stomach Tube to Sprague-Dawley Rats (Experiment BT 958)

Plan of the Experiment

Group	Dose (mg/kg b.w. in olive oil)	Animals	
		Sex	No.
I	1000	M	60
		F	60
		M+F	120
II	250	M	60
		F	60
		M+F	120
III	0[1]	M	60
	(control)	F	60
		M+F	120

[1]Olive oil alone.

Every single dose of MTBE was administered by gavage in 1 ml of extra virgin olive oil, once daily, four days weekly (Monday and Tuesday; Thursday and Friday), for 104 weeks. A daily administration of the compound at the highest dose would not have been tolerated by the rats. The solutions were prepared weekly and maintained at 4°C.

The animals were kept under observation until natural death, under highly standardized housing and diet conditions identical to those used in the BT Laboratory over the last 20 years. In the experiments performed at the BT Cancer Research Center, the animals are usually allowed to live until natural death. By doing so, it is possible, to a feasible extent, to develop all the neoplastic potentialities. Mean daily feed and drinking water consumption were determined once weekly for the first 13 weeks from the start of the experiment, then every 2 weeks, until 112 weeks of age. Individual animal weight was measured once weekly from 8 weeks of age for the first 13 weeks, then every 2 weeks until 112 weeks of age, and

every 8 weeks until the end of the experiment. In order to detect and register all gross lesions, the animals were examined every week for the first 13 weeks, and then every 2 weeks until the end of the experiment.

The biophase of the experiment terminated after 166 weeks, with the death of the last animal at the age of 174 weeks.

Upon death, the animals were submitted to systematic necropsy. Histopathology was routinely performed on skin and subcutaneous tissue, the brain, pituitary gland, Zymbal glands, salivary glands, Harderian glands, cranium (five levels) (with oral and nasal cavities and external and internal ear ducts), tongue, thyroid and parathyroid, pharynx, larynx, thymus and mediastinal lymph nodes, trachea, lung and mainstream bronchi, heart, diaphragm, liver, spleen, pancreas, kidneys and adrenal glands, oesophagus, stomach (fore and glandular), intestine (four levels), bladder, prostate, uterus, gonads, interscapular fat pad, subcutaneous and mesenteric lymph nodes, and any other organ or tissue with pathological lesions.

All organs and tissues were immediately preserved in 70% ethyl alcohol, except bones which were fixed in 10% formalin and then decalcified with a 10% formaldehyde and 20% formic acid in water solution. The normal specimens were trimmed following the standard procedure of the BT Laboratory: i.e., parenchymal organs were trimmed to allow for the largest surface area possible, and hollow organs were dissected through the hilus. The pathological tissue was trimmed to allow for the largest surface with normal adjacent tissues. The trimmed specimens were processed as paraffin blocks, and 3–5 micron sections of every specimen were obtained. Sections were routinely stained with haematoxylin-eosin. Specific stainings were performed when needed. All slides were examined microscopically by the same group of pathologists; a senior pathologist reviewed all the tumors and any other lesion of oncological interest. All pathologists followed the same criteria of histopathological evaluation and classification.

Two statistical methods were used for the analysis of tumor incidence. The first method is a prevalence analysis for nonlethal tumors and is described in Hoel and Walburg (1972). The Leydig cell testicular tumors given in Table 5 were considered nonlethal. The second method assumes lethality of the tumor and the statistical method is a log-ranked test and is described in Mantel (1966) and Cox (1972). This was used for the lymphomas/leukemias on Table 6.

RESULTS

There were no differences in water and feed consumption among the animals of the treated and control groups of both sexes (Graphs 1–4).

There were no relevant differences in mean body weights of treated and control group male and female rats (Graphs 5 and 6). The survival was the same among male rats of the treated and control groups up to 88 weeks of age (80 weeks from the start of the treatment); then there was a higher survival of the males treated at the higher dose (Graph 7). A dose-response

decrease in survival was observed among female rats from 24 weeks of age (16 weeks from the start of treatment) (Graph 8).

No evident behavioral changes were observed in MTBE-treated animals.

No relevant nononcological changes were detected by gross inspection and histological examination.

The benign and malignant tumors observed among male and female rats are listed in Table 4.

The incidence of testicular Leydig cell tumors increased sharply in rats treated with the higher dose (Table 5). The increase was statistically significant at the p=0.05 level.

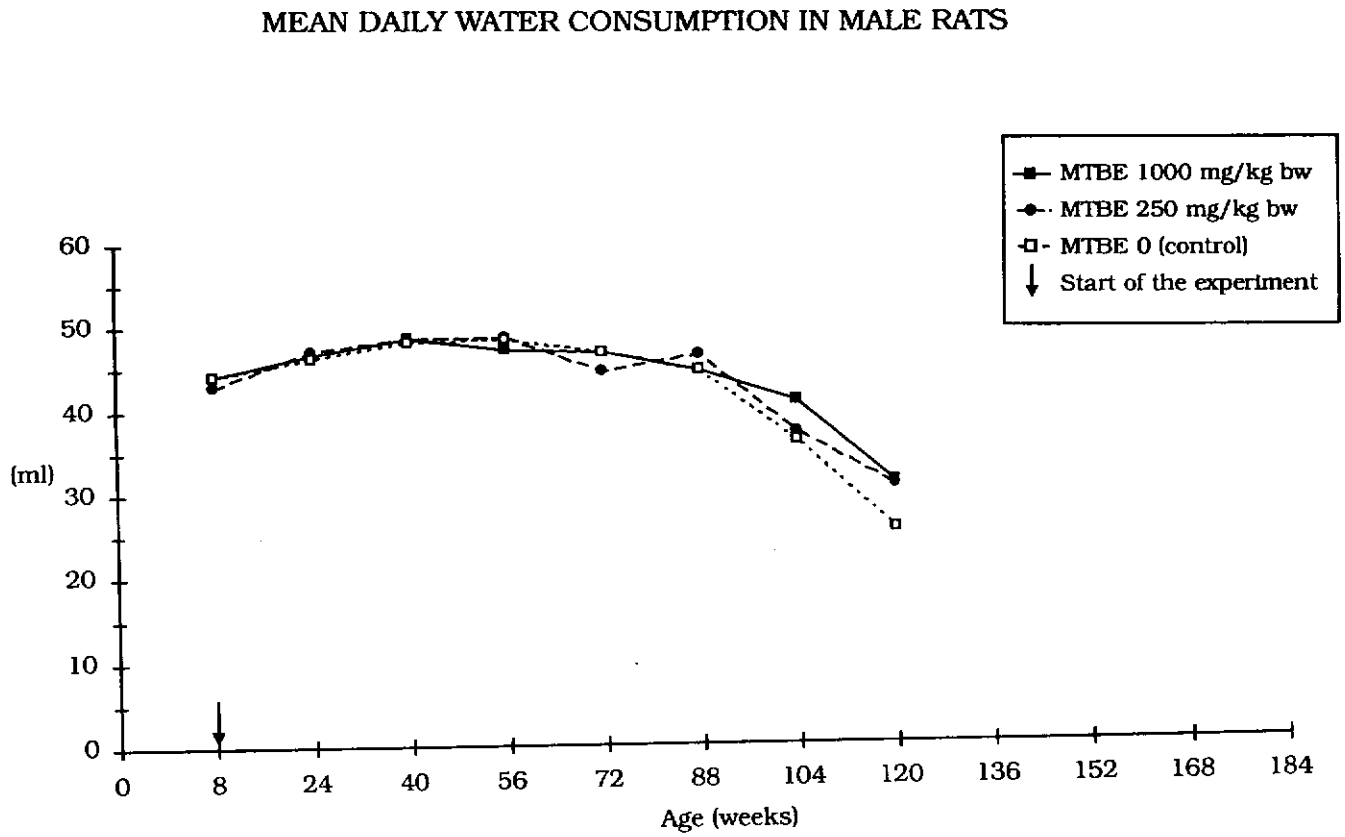
MTBE causes a dose-related increase in lymphomas and leukemias among female rats (Table 6). The fluctuations in the expected incidence of these neoplasias among female Sprague-Dawley rats in our historical controls is currently in a range below 10%. The incidence of lymphomas and leukemias in male Sprague-Dawley rats, which is slightly lower among animals exposed to the higher dose of MTBE, is within the range of the expected fluctuations in the historical controls. The increase in the females was highly significant ($p < 0.01$) at the highest dose level and marginally significant ($p < 0.01$) at the mid-dose level.

An increase in dysplastic proliferation of lymphoreticular tissue was found in female rats treated with MTBE at both doses (Table 7). The term dysplastic proliferation of lymphoreticular tissue defines a hyperplastic lymphoid tissue, at various body sites, in which atypical lymphoid cells (usually lymphoimmunoblasts), isolated and/or aggregated in small clusters, are observed. The incidence of this type of change is higher among animals treated at the lower dose. This apparently paradoxical effect is probably due to the fact that more of these dysplastic proliferations might have developed into lymphomas and leukemias among female rats treated at the higher dose.

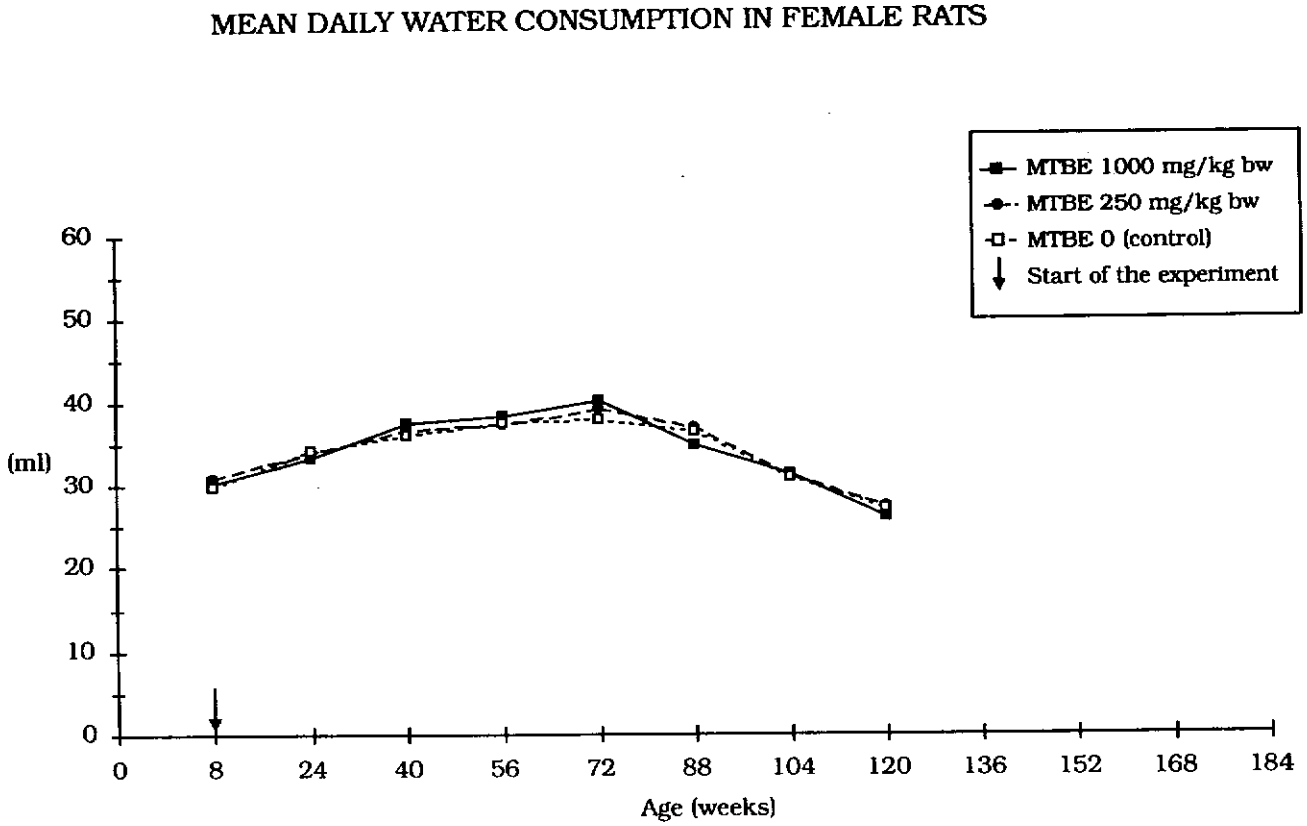
The incidence of all the other tumors at various sites was in the range of the expected fluctuations. A dose-related decrease in mammary fibromas and fibroadenomas, and in pituitary adenoma and tumors of adrenal glands was observed in treated female animals; this effect may be due to the increased dose-related early mortality caused by the treatment with MTBE since the incidence of these tumors is age-dependent in our colony of Sprague-Dawley rats. An increased incidence of uterine sarcomas was observed in female rats exposed to the lower dose.

CONCLUSIONS AND DISCUSSION

MTBE is rapidly absorbed following oral exposure (Bio-Research Laboratories Limited, 1990a). In the tested experimental conditions, MTBE, administered by stomach tube, caused an increase in Leydig interstitial cell tumors of testes in male rats; in female rats, an increase in lymphomas and leukemias was noted. Moreover, an increase in uterine sarcomas was found in female rats treated with the lower dose. Regarding this last finding, it is interesting to note



GRAPH 1. Long-term carcinogenicity bioassays on METHYL-TERTIARY-BUTYL ETHER (MTBE), administered by stomach tube to Sprague-Dawley rats (Experiment BT 958).



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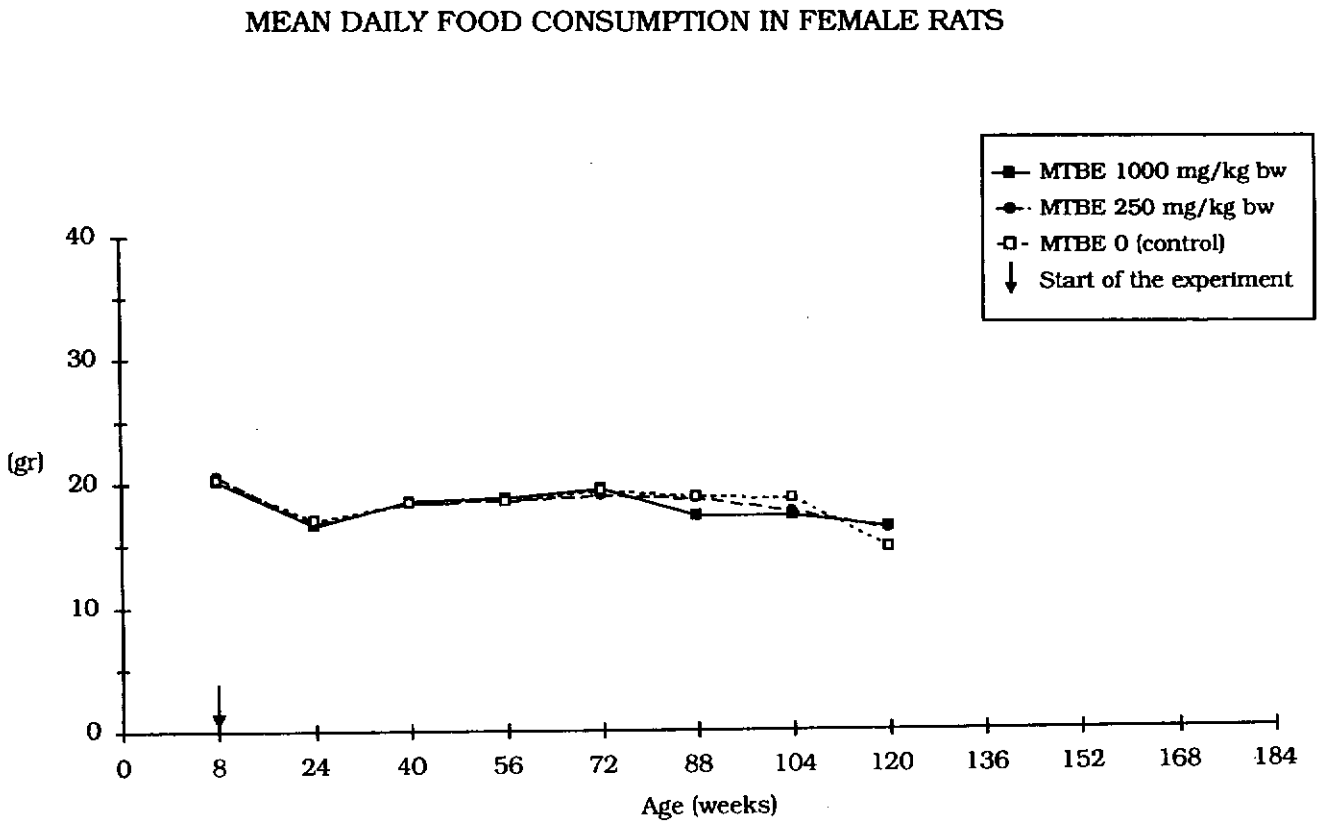

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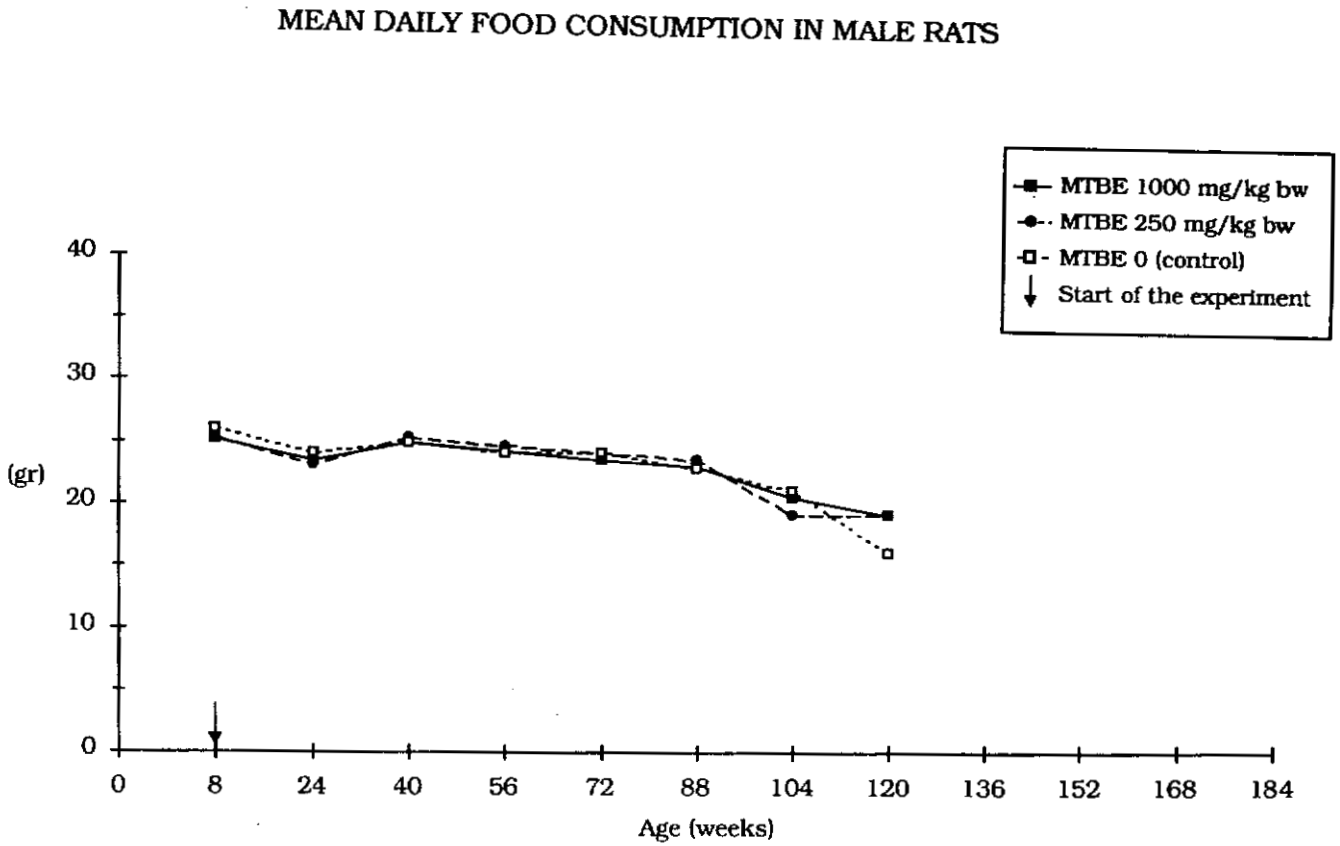

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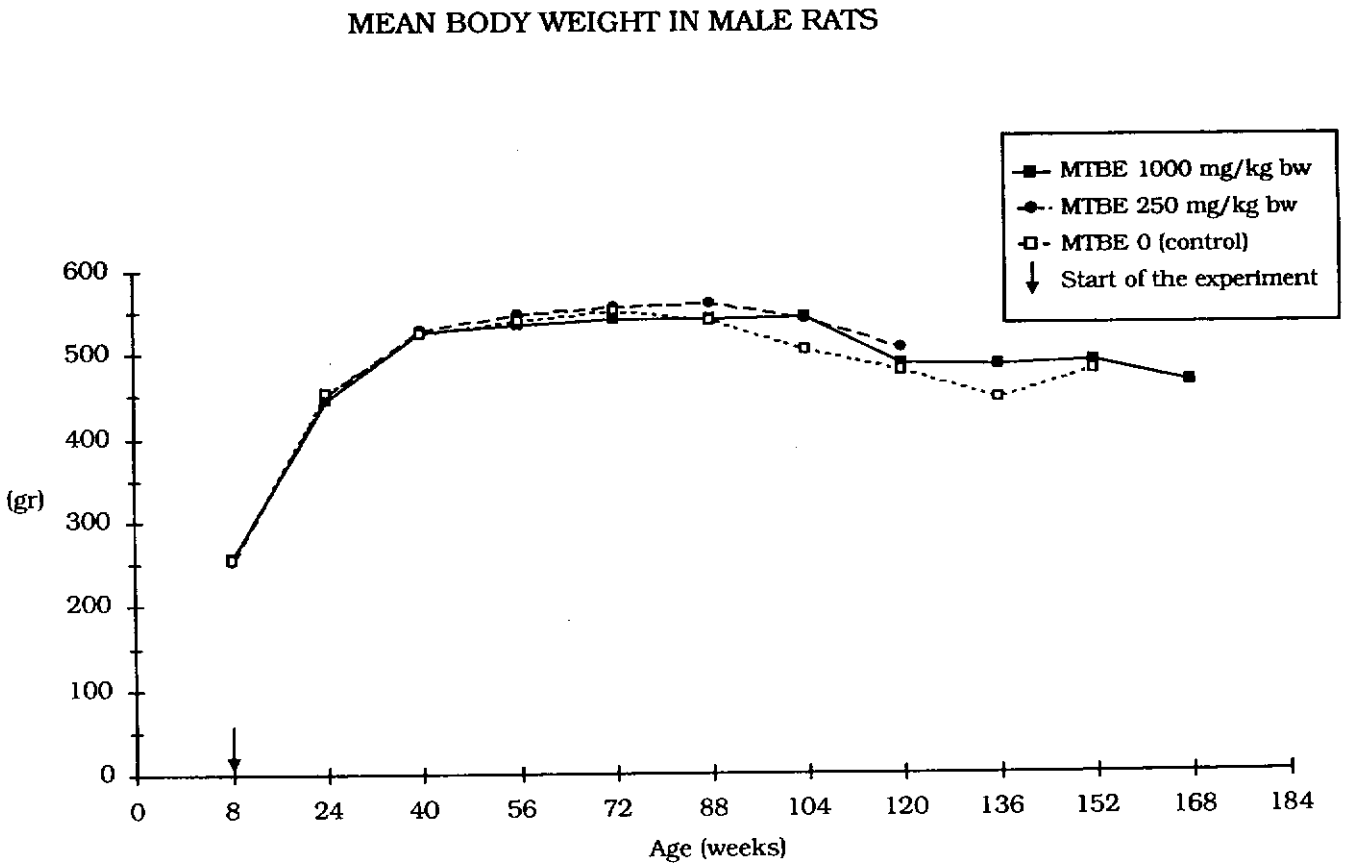
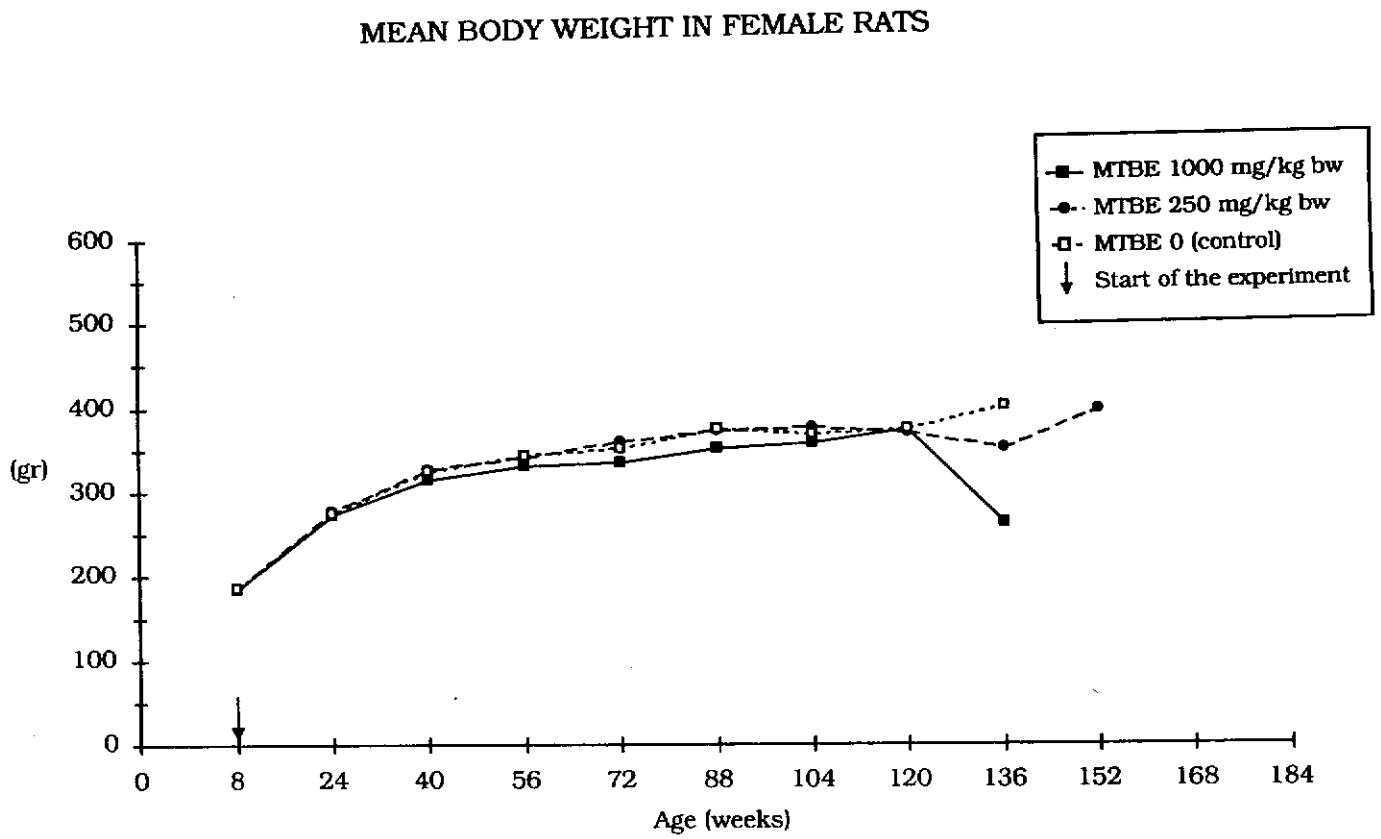
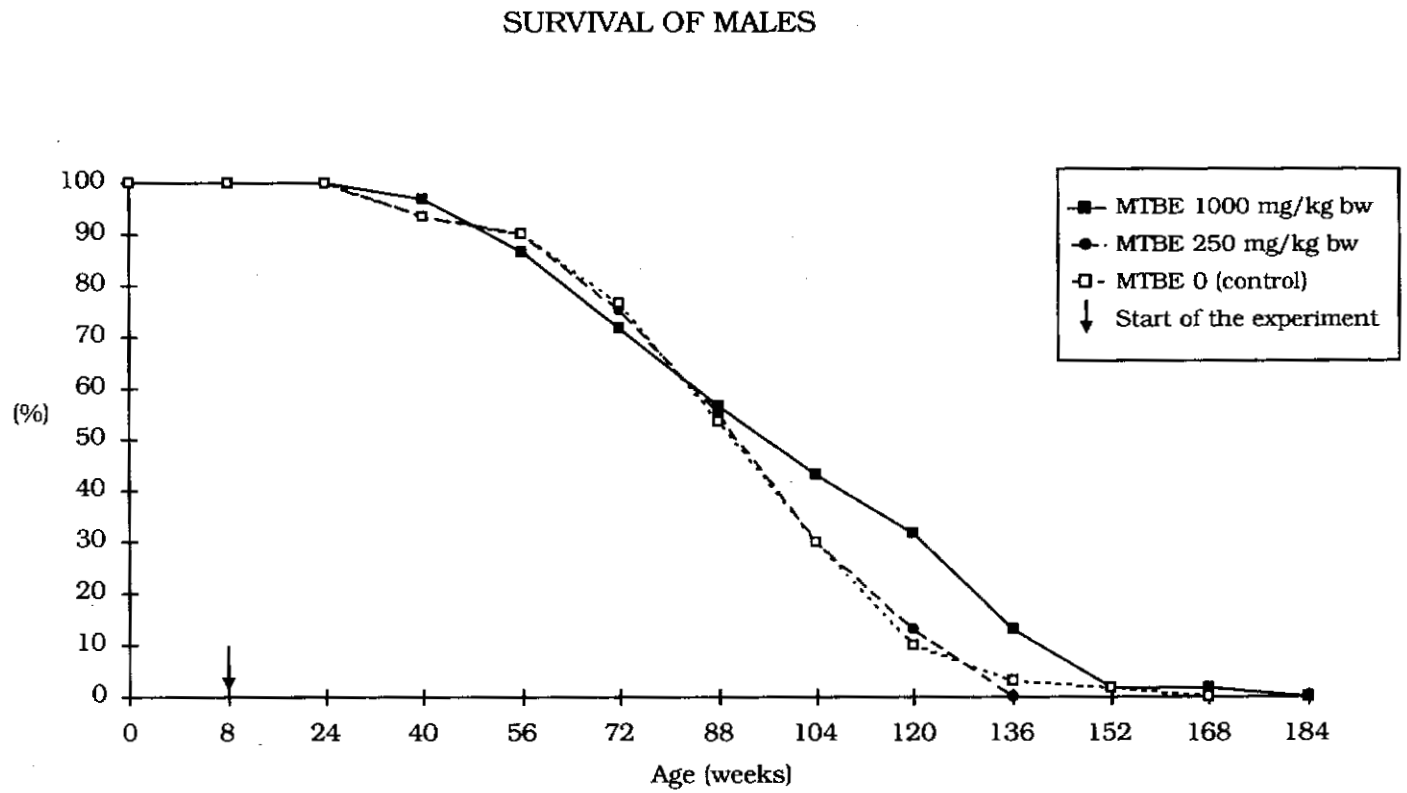


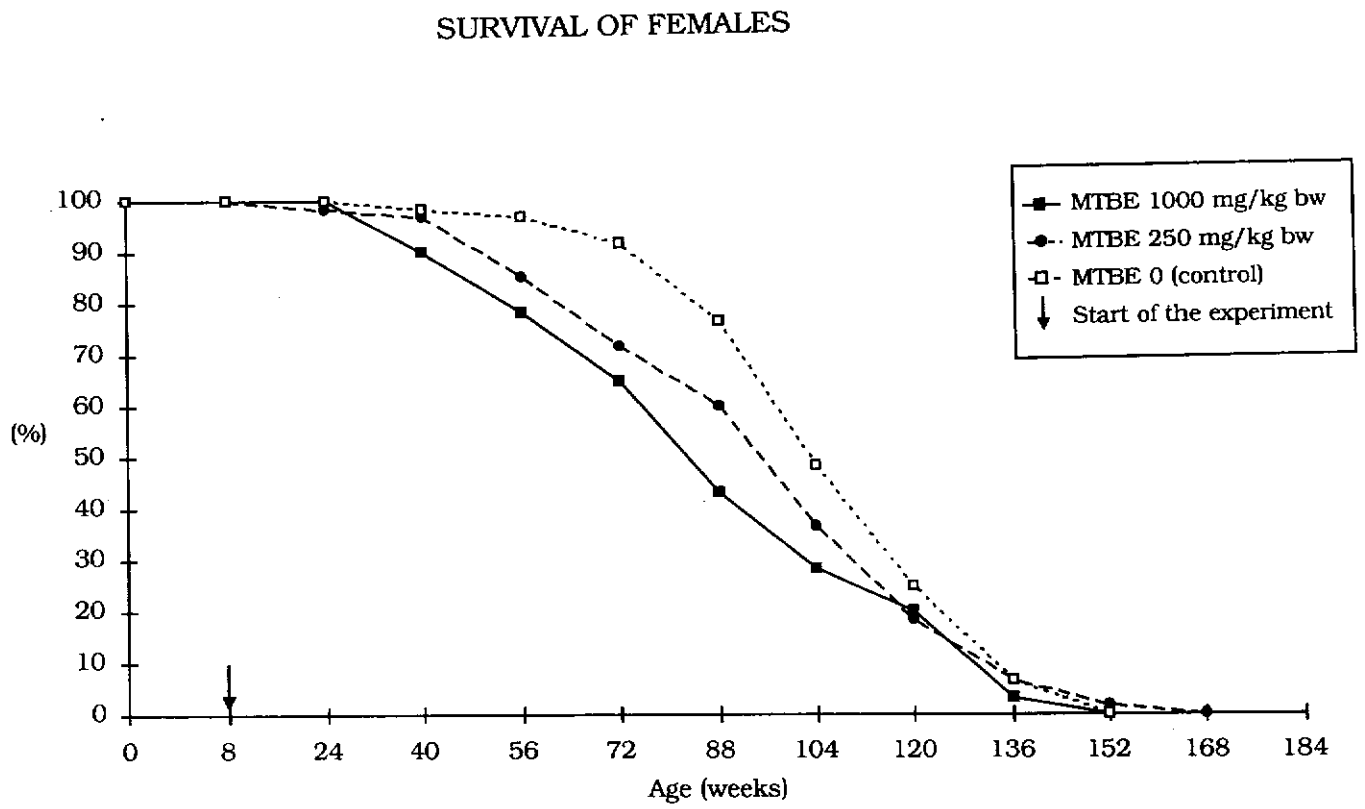
GRAPH 5. Long-term carcinogenicity bioassays on METHYL-TERTIARY-BUTYL ETHER (MTBE), administered by stomach tube to Sprague-Dawley rats (Experiment BT 958).



GRAPH 6. Long-term carcinogenicity bioassays on METHYL-TERTIARY-BUTYL ETHER (MTBE), administered by stomach tube to Sprague-Dawley rats (Experiment BT 958).



GRAPH 7. Long-term carcinogenicity bioassays on METHYL-TERTIARY-BUTYL ETHER (MTBE), administered by stomach tube to Sprague-Dawley rats (Experiment BT 958).



GRAPH 8. Long-term carcinogenicity bioassays on METHYL-TERTIARY-BUTYL ETHER (MTBE), administered by stomach tube to Sprague-Dawley rats (Experiment BT 958).

TABLE 4. Long-Term Carcinogenicity Bioassays on METHYL-TERTIARY-BUTYL ETHER (MTBE), Administered by Stomach Tube to Sprague-Dawley Rats (Experiment BT 958)

Incidence of Various Types of Benign and Malignant Tumors among Male Sprague-Dawley Rats

(Part I)

Site	Histotype	Groups					
		I: 1000 mg/kg b.w.		II: 250 mg/kg b.w.		III: 0[1] (control)	
		No.	%	No.	%	No.	%
Subcutaneous tissue	Fibroma	1	1.7	0	–	1	1.7
	Lipoma	1	1.7	0	–	0	–
	Liposarcoma	1	1.7	0	–	0	–
Mammary gland	Fibroma	3	5.0	2	3.3	1	1.7
	Fibrolipoma	0	–	1	1.7	0	–
	Lipoma	0	–	0	–	1	1.7
	Adenocarcinoma	1	1.7	1	1.7	1	1.7
	Liposarcoma	0	–	0	–	1	1.7
Zymbal gland	Carcinoma	2	3.3	1	1.7	3	5.0
Ear duct	Carcinoma	3	5.0	3	5.0	2	3.3
Nasal cavities	Olfactory neuroblastoma	0	–	0	–	1	1.7
Oral cavity, lips, and tongue	Carcinoma	2	3.3	1	1.7	0	–

[1]Olive oil alone.

TABLE 4. Long-Term Carcinogenicity Bioassays on METHYL-TERTIARY-BUTYL ETHER (MTBE), Administered by Stomach Tube to Sprague-Dawley Rats (Experiment BT 958)

Incidence of Various Types of Benign and Malignant Tumors among Male Sprague-Dawley Rats

(Part II)

Site	Histotype	Groups					
		I: 1000 mg/kg b.w.		II: 250 mg/kg b.w.		III: 0[1] (control)	
		No.	%	No.	%	No.	%
Larynx	Squamous cell carcinoma	0	–	1	1.7	0	–
Lung	Fibroangioma	0	–	0	–	1	1.7
	Adenocarcinoma	0	–	0	–	1	1.7
Liver	Fibroangioma	0	–	1	1.7	0	–
	Hepatocarcinoma	1	1.7	2	3.3	0	–
Pancreas	Islet cell adenoma	5	8.3	2	3.3	4	6.7
	Exocrine adenocarcinoma	1	1.7	0	–	0	–
Kidneys	Adenoma	2	3.3	1	1.7	1	1.7
Prostate	Adenocarcinoma	0	–	1	1.7	0	–
Testes[2]	Leydig cell tumor	11	18.3	2	3.3	2	3.3

[1]Olive oil alone.
[2]See Table 5.

TABLE 4. Long-Term Carcinogenicity Bioassays on METHYL-TERTIARY-BUTYL ETHER (MTBE), Administered by Stomach Tube to Sprague-Dawley Rats (Experiment BT 958)

Incidence of Various Types of Benign and Malignant Tumors Among Male Sprague-Dawley Rats

(Part III)

Site	Histotype	Groups					
		I: 1000 mg/kg b.w.		II: 250 mg/kg b.w.		III: 0[1] (control)	
		No.	%	No.	%	No.	%
Pituitary gland	Adenoma	10	16.7	9	15.0	11	18.3
Thyroid gland	C-cell adenoma	1	1.7	2	3.3	4	6.7
	C-cell carcinoma	1	1.7	0	–	0	–
Adrenal glands	Cortical adenoma	0	–	1	1.7	1	1.7
	Pheocromocytoma	20	33.3	13	21.7	13	21.7
	Pheocromoblastoma	1	1.7	1	1.7	0	–
Central nervous system							
– Brain	Oligodendroglioma	3	5.0	2	3.3	3	5.0
	Neuroblastoma	0	–	1	1.7	0	–
– Meninges	Meningioma	0	–	1	1.7	0	–
Bones							
– Cranium	Osteosarcoma	1	1.7	2	3.3	3	5.0

[1]Olive oil alone.

TABLE 4. Long-Term Carcinogenicity Bioassays on METHYL-TERTIARY-BUTYL ETHER (MTBE), Administered by Stomach Tube to Sprague-Dawley Rats (Experiment BT 958)

Incidence of Various Types of Benign and Malignant Tumors among Male Sprague-Dawley Rats

(Part IV)

Site	Histotype	Groups					
		I: 1000 mg/kg b.w.		II: 250 mg/kg b.w.		III: 0[1] (control)	
		No.	%	No.	%	No.	%
Soft tissues	Liposarcoma	0	–	1	1.7	0	–
Heart	Malignant Schwannoma	0	–	0	–	1	1.7
Subcutaneous lymph nodes	Fibroangioma	0	–	1	1.7	0	–
Haemolymphoreticular tissues[3,4]	Lymphomas and leukemias	7	11.7	9	15.0	10	16.7

[1]Olive oil alone.
[3]Including subcutaneous lymph nodes.
[4]See Table 6.

TABLE 4. Long-Term Carcinogenicity Bioassays on METHYL-TERTIARY-BUTYL ETHER (MTBE), Administered by Stomach Tube to Sprague-Dawley Rats (Experiment BT 958)

***Incidence of* Various Types of Benign and Malignant Tumors among Female Sprague-Dawley Rats**

(Part V)

Site	Histotype	Groups					
		I: 1000 mg/kg b.w.		II: 250 mg/kg b.w.		III: 0[1] (control)	
		No.	%	No.	%	No.	%
Skin	Dermatofibroma	0	–	1	1.7	1	1.7
	Squamous cell carcinoma	0	–	0	–	1	1.7
Subcutaneous tissue	Lipoma	1	1.7	0	–	0	–
Mammary gland	Fibroma and fibroadenoma	16	26.7	27	45.0	40	66.7
	Fibrolipoma	1	1.7	0	–	0	–
	Adenocarcinoma	2	3.3	6	10.0	4	6.7
	Carcinosarcoma	0	–	1	1.7	0	–
Zymbal gland	Carcinoma	2	3.3	0	–	2	3.3
Ear duct	Acanthoma	1	1.7	0	–	0	–
	Carcinoma	0	–	0	–	4	6.7
Oral cavity, lips, and tongue	Acanthoma	1	1.7	0	–	2	3.3
	Odontoma	0	–	0	–	1	1.7
	Carcinoma	0	–	2	3.3	1	1.7

[1]Olive oil alone.

TABLE 4. Long-Term Carcinogenicity Bioassays on METHYL-TERTIARY-BUTYL ETHER (MTBE), Administered by Stomach Tube to Sprague-Dawley Rats (Experiment BT 958)

Incidence of Various Types of Benign and Malignant Tumors among Female Sprague-Dawley Rats

(Part VI)

Site	Histotype	Groups					
		I: 1000 mg/kg b.w.		II: 250 mg/kg b.w.		III: 0[1] (control)	
		No.	%	No.	%	No.	%
Larynx	Squamous cell carcinoma	0	–	1	1.7	0	–
Pharynx	Squamous cell carcinoma	1	1.7	0	–	0	–
Lung	*Fibroangioma*	0	–	1	1.7	0	–
Stomach							
– Forestomach	Acanthoma	3	5.0	2	3.3	1	1.7
Liver	Cholangiofibroma	0	–	1	1.7	0	–
Pancreas	Exocrine adenoma	1	1.7	2	3.3	1	1.7
	Islet cell adenoma	2	3.3	1	1.7	1	1.7
Kidneys	Lipoma	0	–	0	–	1	1.7

[1] Olive oil alone.

TABLE 4. Long-Term Carcinogenicity *Bioassays on* METHYL-TERTIARY-BUTYL ETHER (MTBE), Administered by Stomach Tube to Sprague-Dawley Rats (Experiment BT 958)

Incidence of Various Types of Benign and Malignant Tumors among Female Sprague-Dawley Rats

(Part VII)

Site	Histotype	Groups					
		I: 1000 mg/kg b.w.		II: 250 mg/kg b.w.		III: 0[1] (control)	
		No.	%	No.	%	No.	%
Ovary	Granulosa cell tumor	1	1.7	3	5.0	0	–
	Granulosa and theca cell tumor	0	–	0	–	1	1.7
	Fibroma	1	1.7	0	–	0	–
Uterus	Polyp	12	20.0	12	20.0	13	21.7
	Fibroma	0	–	2	3.3	1	1.7
	Fibroangioma	1	1.7	0	–	0	–
	Granular cell tumor (Abrikossoff's tumor)	0	–	2	3.3	0	–
	Sarcoma	0	–	5	8.3	1	1.7
Uterus and vagina	Sarcoma	0	–	0	–	1	1.7[2]
Peritoneum	Lipoma	0	–	0	–	3	5.0
Pituitary gland	Adenoma	13	21.6	16	26.7	22	36.7
Thyroid gland	Follicular cell adenoma	2	3.3	0	–	0	–
	C-cell adenoma	3	5.0	10	16.7	5	8.3

[1] Olive oil alone.
[2] This sarcoma was involving both uterus and vagina.

TABLE 4. Long-Term Carcinogenicity Bioassays on METHYL-TERTIARY-BUTYL ETHER (MTBE), Administered by Stomach Tube to Sprague-Dawley Rats (Experiment BT 958)

Incidence of Various Types of Benign and Malignant Tumors among Female Sprague-Dawley Rats

(Part VIII)

Site	Histotype	Groups					
		I: 1000 mg/kg b.w.		II: 250 mg/kg b.w.		III: 0[1] (control)	
		No.	%	No.	%	No.	%
Adrenal glands	Cortical adenoma	3	5.0	6	10.0	5	8.3
	Pheocromocytoma	10	16.7	11	18.3	18	30.0
	Cortical adenocarcinoma	0	–	4	6.7	3	5.0
	Pheocromoblastoma	0	–	0	–	2	3.3
Central nervous system							
– Brain	Oligodendroglioma	0	–	0	–	1	1.7
– Meninges	Meningioma	0	–	1	1.7	0	–
Bones							
– Cranium	Osteosarcoma	1	1.7	0	–	1	1.7
Mesenteric lymph nodes	Fibroangioma	0	–	1	1.7	0	–
Haemolymphoreticular tissues[3,4]	Lymphoma and leukemia	12	20.0	6	10.0	2	3.3

[1]Olive oil alone.
[3]Including mesenteric lymph nodes.
[4]See Tables 6 and 7.

that an increase in these tumors was found in the same colony of Sprague-Dawley rats following treatment with gasoline (with high content of aromatics) (Maltoni et al., 1995). On the basis of our results, MTBE must be considered an animal carcinogen, and therefore represents a potential carcinogenic risk for humans.

TABLE 5. Long-Term Carcinogenicity Bioassays on Methyl-Tertiary-Butyl Ether (MTBE), Administered by Stomach Tube to Sprague-Dawley Rats (Experiment BT 958)

Results: Leydig Cell Testicular Tumors

Groups No.	Dose (mg/kg b.w. in olive oil)	No. at start	Corrected number[1]	Animals bearing Leydig cell tumors		
				No.	%[2]	%[3]
I	1000	60	32	11	18.3	**34.4**[4]
II	250	60	25	2	3.3	**8.0**
III	0[5] (control)	60	26	2	3.3	**7.7**

[1]Alive male rats at 96 weeks of age, when the first Leydig cell tumor was observed.
[2]Percentages are referred to the number at start.
[3]Percentages are referred to the corrected number.
[4]$p < 0.05$.
[5]Olive oil alone.p

TABLE 6. Long-Term Carcinogenicity Bioassays on Methyl-Tertiary-Butyl Ether (MTBE), Administered by Stomach Tube to Sprague-Dawley Rats (Experiment BT 958)

Results: Lymphomas and Leukemias

Groups No.	Dose (mg/kg b.w. in olive oil)	Male					Female				
		No. at start	Corrected number[1]	Animals bearing Lymphomas and Leukemias			No. at start	Corrected number[2]	Animals bearing Lymphomas and Leukemias		
				No.	%[3]	%[4]			No.	%[3]	%[4]
I	1000	60	58	7	11.7	12.0	60	47	12	20.0	**25.5**[5]
II	250	60	59	9	15.0	15.3	60	51	6	10.0	**11.8**[6]
III	0[7] (control)	60	59	10	16.7	16.9	60	58	2	3.3	**3.4**

[1]Alive male rats at 32 weeks of age, when the first leukemia was observed.
[2]Alive female rats at 56 weeks of age, when the first leukemia was observed.
[3]Percentages are referred to the number at start.
[4]Percentages are referred to the corrected number.
[5]$p < 0.01$.
[6]$p < 0.01$.
[7]Olive oil alone.

TABLE 7. Long-Term Carcinogenicity Bioassays on Methyl-Tertiary-Butyl Ether (MTBE), Administered by Stomach Tube to Sprague-Dawley Rats (Experiment BT 958)

Results: Dysplastic Proliferation of Lymphoreticular Tissues (DPLT) at Various Sites

Groups No.	Dose (mg/kg b.w. in olive oil)	Animals			Animals bearing DPLT		
		Sex	No. at start	Corrected number[1]	No.	%[2]	%[3]
I	1000	F	60	59	9	15.0	**15.3**
II	250	F	60	59	15	25.0	**25.4**
III	0[4] (control)	F	60	60	1	1.7	**1.7**

[1] Alive female rats at 26 weeks of age, when the first DPLT was observed.
[2] Percentages are referred to the number at start.
[3] Percentages are referred to the corrected number.
[4] Olive oil alone.

In another long-term carcinogenicity study (known as the ARCO Study), supported in the United States by a group of producers and users of MTBE, MTBE was tested on both male and female Fischer 344 rats and CD-1 mice. In this study, the compound was tested by inhalation at concentrations of 8000, 3000, 400, and 0 ppm. The results of this unpublished study showed MTBE to be carcinogenic (Burleigh-Flayer et al., 1992; Chun et al., 1992); the compound increased the incidence of a rare type of kidney tumor (renal tubular adenomas and carcinomas) in male rats exposed to high-dose and particularly to mid-dose. This was statistically significant when compared to concurrent controls. Further, a mid-dose-related increase (still statistically significant) in "interstitial cell adenoma of testes" was noted in male rats. This increase was difficult to evaluate because of the high background of the spontaneous incidence of those tumors in their historical controls (Haseman et al., 1985). The results of the ARCO study also showed that MTBE causes an increase in the incidence of hepatocellular adenomas in female mice. In the ARCO rat study there was a high and apparently dose-related incidence of noncancer renal toxicity in both sexes, an effect which was not observed in our experiment.

It is interesting to note that in the studies conducted by ARCO and at the BT Laboratory, MTBE was found to increase the incidence of Leydig cell testicular tumors. Our results and the results of the ARCO study, although different, converge in exposing the carcinogenic potential of MTBE. Moreover, the combined results of the two studies appear to indicate that MTBE *is a trans-species, multistrain, multisite carcinogen.*

The most striking indication of MTBE carcinogenicity is the increase in lymphomas and leukemias among female rats observed in the BT bioassays. There are not yet sufficient data to define the mechanisms by which MTBE acts as a leukemogen, although some available information may prove helpful. MTBE was reported to metabolize to tertiary butyl alcohol and formaldehyde by microsomal demethylation (Savolainen et al., 1985; Brady et al., 1990). On the other hand, preliminary results of long-term carcinogenicity bioassays of formaldehyde administered by drinking water showed that this compound increases the incidence of lymphomas and leukemias in both males and females (Soffritti et al., 1989). These early results were more consistently confirmed by our complete and conclusive data (Soffritti et al., 1995). Pharmacokinetic studies have shown sex differences which deserve further investigation; after exposure, a decreased plasma concentration of MTBE was observed in female rats (Bio-Research Laboratories Limited, 1990b). This result suggests that in females, MTBE undergoes a more rapid/intense metabolization. All these data seem to point to the formaldehyde transformation of MTBE as a probable crucial step in its leukemogenic effect.

On the basis of these results of the BT experiments, and of the other available information, MTBE must be considered as a potential human carcinogen. Therefore, its use constitutes a worldwide public health problem. This problem is aggravated by the fact that the addition of MTBE to gasoline produces increased levels of formaldehyde in the exhaust (Stump et al., 1990).

Further investigation is needed to better define and quantitatively assess the carcinogenicity of MTBE. Even today, however, the use of MTBE in gasoline must be reconsidered in view of the available data. Measures of prevention must be undertaken in the scenarios of heavier exposure.

Additionally, in view of the fact that MTBE is found in the drinking water of some localities, this study provides a better assessment of risk by exposure in drinking water than the unpublished inhalation studies. Thus, the risk to public health from water contaminated by MTBE should be alleviated at once.

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