

Comprehensive long-term experimental project of carcinogenicity bioassays on gasoline oxygenated additives: plan and first report of results from the study on ethyl-tertiary-butyl ether (ETBE) ^{(a) (b)}

C. Maltoni, F. Belpoggi, M. Soffritti and F. Minardi

Cancer Research Centre, European Ramazzini Foundation of Oncology and Environmental Sciences, Bologna, Italia

C. Maltoni, F. Belpoggi, M. Soffritti and F. Minardi: Comprehensive long-term experimental project of carcinogenicity bioassays on gasoline oxygenated additives: plan and first report of results from the study on ethyl-tertiary-butyl ether (ETBE). Eur. J. Oncol., 4 (5), 493-508, 1999

C. Maltoni, F. Belpoggi, M. Soffritti e F. Minardi: Il progetto comprensivo di saggi di cancerogenicità a lungo termine degli additivi ossigenati delle benzine: primo resoconto dei risultati sull'etil-ter-butil etere (ETBE). Eur. J. Oncol., 4 (5), 493-508, 1999

Summary

This report illustrates the project of long-term experimental studies on the gasoline oxygenated additives and of gasoline containing several of the same oxygenates, performed by the Cancer Research Centre (CRC) of the European Ramazzini Foundation of Oncology and Environmental Sciences (RF). The compounds and mixtures studied by this project are: methyl alcohol, ethyl alcohol, methyl-tertiary-butyl ether (MTBE), ethyl-tertiary-butyl ether (ETBE), tert-amyl-methyl ether (TAME) and di-isopropyl ether (DIPE), as well as gasoline containing methyl alcohol, ethyl alcohol, MTBE and ETBE. All experiments were performed on Sprague-Dawley rats from the CRC/RF colony, exposed to test materials by ingestion (stomach intubation in extra virgin olive oil solution, or in drinking water) and kept under control until spontaneous death. The report also presents the first results of the study on ETBE. The compound was administered to groups of 120 rats (60 males and 60 females) at a daily dose of 1000, 250, and 0 (in olive oil) mg/kg b.w., for 4 days weekly, over 104 weeks. In the tested experimental conditions ETBE causes an increase in: 1) total malignant tumours (more evident in females); 2) total oncological lesions of the mouth (more evident among males); 3) total oncological lesions of the forestomach

Riassunto

In questo resoconto viene presentato il progetto di studi sperimentali a lungo termine sulla cancerogenicità degli additivi ossigenati delle benzine e di benzine contenenti alcuni degli stessi ossigenati, condotto presso il Centro di Ricerca sul Cancro (CRC) della Fondazione Europea di Oncologia e Scienze Ambientali "B. Ramazzini" (FR). Con questo progetto si sono voluti studiare i seguenti composti/miscele: l'alcool metilico, l'alcool etilico, il metil-ter-butil etere (MTBE), l'etil-ter-butil etere (ETBE), il ter-amil-metil etere (TAME) ed il di-isopropil etere (DIPE), e benzine contenenti alcool metilico, alcool etilico, MTBE ed ETBE. Tutti gli esperimenti sono stati condotti su ratti Sprague-Dawley della colonia del CRC/FR, esposti ai materiali in studio per via ingestiva (mediante gavaggio in soluzione di olio di oliva, o con l'acqua da bere), e tenuti sotto controllo fino a morte spontanea. Nel resoconto vengono anche riferiti i primi risultati dello studio sull'ETBE. Il composto è stato somministrato a gruppi di 120 ratti (60 maschi e 60 femmine), alle dosi di 1000, 250 e 0 (in olio di oliva) mg/kg p.c., per giorno, per 4 giorni alla settimana, per 104 settimane. Nelle condizioni sperimentali saggate l'ETBE determina, negli animali trattati, un aumento di: 1) tumori maligni totali (più evidente nelle femmine); 2) lesioni di interesse oncologico totali della bocca (soprattutto evidenti nei maschi); 3) lesioni di interesse oncologico totali del pre-stomaco nei maschi e, più in specifico, carcinomi squamocellulari sia nei maschi che nelle femmine, esposti alla concentrazione più alta di ETBE; 4) tumori maligni dell'utero (in particolare sarcomi) nelle femmine esposte alla concentrazione più bassa; e 5) neoplasie emolinfocitiche (linfomi e leucemie, in particolare linfomi linfocitici). Non è stata riscontrata una correlazione tra effetti neoplastici e concentrazione di ETBE: questo risultato è probabilmente

^(a) This manuscript was updated in July 1999

^(b) This research has been supported by the Regional Agency for Prevention and Environment (Agenzia Regionale Prevenzione e Ambiente = ARPA), of the Emilia Romagna Region

Indirizzo/Address: Cesare Maltoni, Centro di Ricerca sul Cancro, Fondazione Europea di Oncologia e Scienze Ambientali "B. Ramazzini", Castello di Bentivoglio, 40010 Bentivoglio (BO), Italia

in males and, more specifically, squamocellular carcinomas in both males and females, exposed to the lower concentration of ETBE; 4) malignant uterine tumours (in particular sarcomas) in the females exposed to the lower concentration; and 5) haemolymphoreticular neoplasias (lymphomas and leukaemias), in particular lymphoimmunoblastic lymphomas. No dose-response relationship between neoplastic effects and ETBE concentrations was found: these results may be explained (at least partly) by the higher mortality in the group treated with the higher dose.

Key words: gasoline oxygenated additives, ethyl-tertiary-butyl ether (ETBE), rats, carcinogenesis

Introduction

Gasoline oxygenated additives: the present situation

The gasoline oxygenated additives being proposed at the moment include: methyl alcohol, ethyl alcohol, methyl-tertiary-butyl ether (MTBE), ethyl-tertiary-butyl ether (ETBE), di-isopropyl ether (DIPE) and tertiary-amyl-methyl ether (TAME).

Their use in gasoline is intended to improve the combustion process and, more specifically, to significantly reduce motor vehicle CO emission, especially at low temperatures during winter months. It is also intended to dilute toxic compounds in fuels.

On gasoline oxygenated additives there is a convergence of interests by strong economic powers: agriculture, petrol and petrol-chemical industries and the automobile industry.

The US has been the first country to employ oxygenates widely in fuels. The Clean Air Act amendment of 1990 required the use of oxygenates as well as a 15% reduction in toxic components in gasoline.

Congress brought in the use of oxygenated fuels in nine big metropolitan areas (those with the dirtiest air, including New York, Philadelphia, Chicago, Los Angeles, and Houston); other communities voluntarily took up the scheme.

In the US most oil companies choose MTBE, with the aim of: enhancing octanes, cutting motor emission, increasing oxygen contents and therefore reducing the presence of carbon monoxide in motor emission, and diluting toxic components like benzene and toluene. The use of MTBE was recommended in terms of environmental protection and health safety (API, 1988; US EPA, 1988).

MTBE ($C_5H_{12}O$) is produced by reacting isobutylene with methanol, and is a clear, colourless liquid, with a characteristic terpene odour. It was already used in the US as a gasoline additive in the '80s. During the period 1984-1988 the percent of MTBE-containing gasoline increased from 8 to 22%. In Italy the industrial production of MTBE began in 1985.

At present MTBE makes up 3-5% of the national US gasoline supply, equivalent to 85% of oxygenate needs; ethanol and ETBE practically make up for the rest. MTBE in the US is added to gasoline at concentrations of up to 15% in the cooler states. Its use has gone on expanding in other countries in recent years but the US is by far the major consumer. Presently MTBE is produced world-wide at the rate of about 20 million tons per year.

spiegato (almeno parzialmente) dalla più alta mortalità nel gruppo trattato con la dose più alta.

Parole chiave: additivi ossigenati delle benzine, etil-ter-butil etere (ETBE), ratto, cancerogenesi

When the practice was started (recommended or prescribed) of oxygenating gasolines in general, and in particular with MTBE, there were no precise data on their unwanted side effects on the environment and health; in other words the widespread use of the MTBE and other oxygenates in gasoline preceded any adequate environmental and toxicological testing. Such a policy is in complete contrast with the scientific orientation which recommends adequate studies to expose and assess risks from agents destined to be heavily introduced in the human environment, before they are produced and used. More specifically, there was no available scientific information on the carcinogenicity of these compounds. In recent years people have started to complain about the smell of MTBE at petrol station pumps and the complaints seem to correlate with "winter-time gasoline" which contains a higher concentration of MTBE.

When MTBE-containing gasoline leaks from underground tanks or pipelines or spills, MTBE dissolves in water and may flow into wells, making the water unusable for human purposes.

MTBE has been shown to be carcinogenic in two different experimental sets of studies. In one such study conducted by us, it was shown that MTBE causes in Sprague-Dawley rats an increase in lymphomas/leukaemias (mainly due to lymphoimmunoblastic lymphomas) in the female, and in the male an increase in interstitial cell adenomas of the testis (Belpoggi, Soffritti and Maltoni, 1995; Belpoggi *et al.*, 1997; Belpoggi, Soffritti and Maltoni, 1998). In another study, promoted by producers and users, MTBE was found to cause an increase in hepatocellular adenomas in female CD1 mice, and an increase in renal tubular adenomas and carcinomas in male Fischer 344 rats (data released by Burleigh-Flayer, Chun and Kintish, 1992; Chun, Burleigh-Flayer and Kintish, 1992).

Because of MTBE's carcinogenic potential, air pollution and contamination of the water supply, two steps have been taken in the US.

- 1) In March 1999, California ordered oil companies to phase MTBE out by 2002 (under the Clean Air Act, subject to veto by the US Environmental Protection Agency – US EPA –, California can introduce its own clean air policies because pollution in Los Angeles is worse than in the rest of the country).
- 2) In July 27, 1999, there was a statement by Carol M. Browner, US EPA Administrator, based on findings by EPA's Blue Ribbon MTBE Panel, which we report here in full: "On November 30, 1998, I appointed a Blue Ribbon Panel of leading ex-

perts to investigate concerns raised by the discovery of MTBE, a gasoline additive, in some water supplies. I want to congratulate and thank each member of the Panel for their excellent work, beginning with the Chairman, Dr. Daniel Greenbaum of the Health Effects Institute of Cambridge, MA. The Panel recognized the significant benefits of reduced auto emission and improved air quality from cleaner burning gasoline and the role oxygenates, like MTBE and ethanol, have played. But the Panel also found that when gasoline leaks and spills from sources such as underground storage, tanks and gas cans, MTBE can, in fact, pose risks to water supplies. I stated when the Panel was assembled that a major goal was to protect public health and the environment by ensuring that Americans have both cleaner air and water, and never one at the expense of the other. And that is what EPA intends to do. The recommendations I received from the Panel confirm EPA's belief that we must begin to significantly reduce the use of MTBE in gasoline as quickly as possible without sacrificing the gains we've made in achieving cleaner air. EPA is committed to working with Congress to provide a targeted legislative solution that maintains our air quality gains and allows for the reduction of MTBE, while preserving the important role of renewable fuels like ethanol. In addition, EPA will improve gasoline leak protection and remediation programs and provide the states with maximum flexibility under current law that will make it easier to voluntarily reduce MTBE and use cleaner gasolines with other additives. These actions will ensure that millions of Americans will contribute to breath healthier air in their communities while also receiving improved protection of their water supplies".

The project of the Cancer Research Centre (CRC) of the European Ramazzini Foundation of Oncology and Environmental Sciences (RF): plan, early results, state-of-the-art and future prospects

A) The plan

In the '80s, at the time when an expansion in the use of MTBE and other oxygenates as gasoline additives came to light, in the absence of any known (to us) programme for assessing the health risk of those products, with particular regard to carcinogenicity, the CRC of Bentivoglio (BT) launched a systematic and integrated project of experimental carcinogenicity bioassays on oxygenated additives, namely: methyl alcohol, ethyl alcohol, MTBE, ETBE, TAME and DIPE, and on gasoline containing methyl alcohol, ethyl alcohol, MTBE and ETBE. The experiments in the project started in sequence.

All experiments were performed on Sprague-Dawley rats from the CRC/RF colony, used by us for more than 30 years and on which there is adequate information as to the expected pathology of historical controls.

The plan of the experiments, their starting dates and the state of the art of the studies are given in Table 1.

B) Results on MTBE

The results of our experiment N. 6 (BT 958) of the CRC/RF project, using MTBE on Sprague-Dawley rats, have shown that the compound produces an increased risk of testis interstitial cell hyperplasia and adenomas in males (Table 2), and greater risks in females of haemolymphoreticular neoplasias

(lymphomas and leukaemias), mainly lymphoimmunoblastic lymphomas, most commonly arising in the lung (Tables 3 and 4), as well as haemolymphoreticular dysplasias (Table 5).

C) State of art and prospects

The foresee reduction or ban on MTBE in the next few years calls for alternative strategies aimed at reducing air pollution. Among these strategies the use of substitute oxygenates, first of all ETBE, has been proposed. To our knowledge there are no available data or ongoing experiments on the carcinogenic potential of available substitutes. We are therefore working to make available the results of our project as soon as possible to scientific communities, governments and public agencies.

In this report we are publishing the results of experiment N. 7 on ETBE, which were presented at the 1998 Ramazzini Days, held in Carpi, Italy, by the Collegium Ramazzini.

The bioassay on ETBE: experiment N. 7 (BT 959)

ETBE: the compound and its production

ETBE is the "first cousin" competitor of MTBE. It is as good, and probably a little better than MTBE, as an octane enhancer, and it cuts CO emissions.

ETBE is produced from raw materials: ethyl alcohol, a renewable liquid fuel, obtained by fermentation of agricultural products (synthetic alcohol is out of the question since it would be too expensive) and isobutylene produced from natural gas or obtained as a co-product in the oil refining and petrochemical industries. ETBE is highly competitive with MTBE on an economic basis: in fact methyl alcohol prices have risen drastically with much higher feed-stock costs for MTBE producers as a result.

The physical and chemical properties of ETBE are shown in Table 6.

Material and methods

ETBE was supplied by AGIP Petroli (Euron SpA), S. Donato Milanese, Milan, Italy; its purity was higher than 94%. The impurities of the tested compound were as following:

– methyl alcohol	0.01%
– ethyl alcohol	2.88%
– tertiary butyl alcohol (TBA)	1.59%
– MTBE	1.01%
– 2-ethoxy-butane	0.12%
– ter-butyl-isopropyl ether	0.09%
– olefin C8	0.11%

During the experiment, the compound was stored at a temperature of 4°C.

The extra virgin olive oil used as a carrier was provided by Oliaria Toscana (the same oil has been used in CRC/RF Laboratories for 25 years).

Male (M) and female (F) Sprague-Dawley rats from the CRC/RF colony were used. This colony of rats has been employed for various experiments in the BT Laboratory for nearly 25 years. Historical data are available on about 15,000 historical controls, kept under observation for their life-span and submitted to systematic necropsies and standardized histopathological examinations. Data are thus available on the expected incidence of, and

Table 1 - CRC/RF project: long-term (life-span) carcinogenicity bioassays on gasoline oxygenated additives, and of oxygenated additive-containing gasolines, performed on male (M) and female (F) Sprague-Dawley rats: plan of the experiments^(a)

No. (and code) of the experiment (date of start)	Experimental group	Treatment			Sex	Animals		State of the art	
		Dose	Route	Type of administration		Age at start (weeks)	No.		
METHYL ALCOHOL									
1 (BT 7001) (1984)	I	15 mg/l	Ingestion	In drinking water supplied <i>ad libitum</i> for 104 weeks	M	7	50	Concluded; early results published (Soffritti <i>et al.</i> , 1989)	
						F	7		50
	II	0 (control)			M	7	100		
					F	7	100		
2 (BT 960) (1990)	I	20,000 mg/l	Ingestion	In drinking water supplied <i>ad libitum</i> for 104 weeks	M	8	100		Biophase concluded; histopathological evaluation is ongoing
					F	8	100		
	II	5,000 mg/l			M	8	100		
					F	8	100		
	III	500 mg/l			M	8	100		
					F	8	100		
	IV	0 (control)			M	8	100		
					F	8	100		
3 (BT 961) (1993)	I	24 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M	8	60	Biophase concluded; histopathological evaluation is ongoing	
						F	8		60
	II	0 ^(d) (control)			M	8	60		
					F	8	60		
ETHYL ALCOHOL									
4 (BT 6004) (1986)	I	10 % v/v	Ingestion	In drinking water administered <i>ad libitum</i> , life span	M	39 ^(b)	110	Biophase concluded; histopathological evaluation in progress	
						F	(breeders)		110
	II	10 % v/v			M	Embryos ^(c)	30		
					F	(offspring)	39		
	III	0 (control)			M	39 ^(b)	110		
					F	(breeders)	110		
	IV	0 (control)			M	Embryos ^(c)	49		
					F	(offspring)	55		
5 (BT 961) (1993)	I	40 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M	8	60	Biophase concluded; histopathological evaluation in progress	
						F	8		60
	II	0 ^(d) (control)			M	8	60		
					F	8	60		
MTBE									
6 (BT 958) (1988)	I	1,000 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M	8	60	Results verbally presented at the Ramazzini Days in Carpi, Italy, October 1993, and then published (Belpoggi, Soffritti, and Maltoni, 1995; Belpoggi <i>et al.</i> , 1997; Belpoggi, Soffritti and Maltoni, 1998)	
						F	8		60
	II	250 mg/kg b.w.			M	8	60		
					F	8	60		
	III	0 ^(d) (control)			M	8	60		
					F	8	60		

(Table 1 continued)

(Table 1)

No. (and code) of the experiment (date of start)	Experimental group	Treatment			Sex	Animals		State of the art	
		Dose	Route	Type of administration		Age at start (weeks)	No.		
ETBE									
7 (BT 959) (1993)	I	1,000 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M	8	60	Concluded; early results published in this report	
					F	8	60		
	II	250 mg/kg b.w.			M	8	60		
					F	8	60		
	III	0 ^(d) (control)			M	8	60		
					F	8	60		
TAME									
8 (BT 963) (1995)	I	750 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M	8	100	Concluded; awaiting publication	
					F	8	100		
	II	250 mg/kg b.w.			M	8	100		
					F	8	100		
	III	0 ^(d) (control)			M	8	100		
					F	8	100		
DIPE									
9 (BT 964) (1995)	I	1,000 mg/kg b.w.	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M	8	100	Concluded; awaiting publication	
					F	8	100		
	II	250 mg/kg b.w.			M	8	100		
					F	8	100		
	III	0 ^(d) (control)			M	8	100		
					F	8	100		
No. (and code) of the experiment (date of start)	Experimental group	Treatment			Sex	Animals		State of the art	
		Test agent	Dose	Route		Type of administration	Age at start (weeks)		No.
GASOLINE CONTAINING METHYL ALCOHOL									
10 (BT 961) (1993)	I	Gasoline containing 3% methyl alcohol	800	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M	8	60	Biophase concluded; histopathological evaluation in progress
						F	8	60	
	II	Gasoline	768			M	8	60	
						F	8	60	
	III	Control	0 ^(d)			M	8	60	
						F	8	60	
GASOLINE CONTAINING ETHYL ALCOHOL									
11 (BT 961) (1993)	I	Gasoline containing 5% ethyl alcohol	800	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M	8	60	Biophase concluded; histopathological evaluation in progress
						F	8	60	
	II	Gasoline	768			M	8	60	
						F	8	60	
	III	Control	0 ^(d)			M	8	60	
						F	8	60	

(Table 1 continued)

(Table 1)

No. (and code) of the experiment (date of start)	Experimental group	Treatment				Animals			State of the art
		Test agent	Dose	Route	Type of administration	Sex	Age at start (weeks)	No.	
GASOLINE CONTAINING MTBE									
12 (BT 961) (1993)	I	Gasoline containing 15% MTBE	800	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M	8	60	Biophase concluded; histopathological evaluation in progress
						F	8	60	
	II	Control	0 ^(a)			M	8	60	
						F	8	60	
GASOLINE CONTAINING ETBE									
13 (BT 962) (1993)	I	Gasoline containing 15% ETBE	800	Ingestion	By stomach tube, in olive oil, 4 days weekly, for 104 weeks	M	8	60	Biophase concluded; histopathological evaluation in progress
						F	8	60	
	II	Control	0 ^(a)			M	8	60	
						F	8	60	

^(a) Experiments 3, 5, 7, 10 and 11, experiments 8 and 9, and experiments 12 and 13, have control groups in common, respectively; experiments 10 and 11 have groups treated with gasoline alone in common

^(b) From 7 days before mating

^(c) From conception

^(d) Olive oil alone

Table 2 - CRC/RF project: long-term carcinogenicity bioassays on methyl-tertiary-butyl ether (MTBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 958). Results: testicular interstitial cell proliferative and neoplastic pathologies in male rats

Group No.	Dose (mg/kg b.w. in olive oil)	Sex	No. of animals at start	Animals bearing interstitial cell proliferative and neoplastic pathologies											
				Interstitial cell hyperplasias ^(a)				Interstitial cell adenomas							
				Total		Focal		Total		I single histocytotype		Mixed histocytotype		Multifocal	
				No.	% ^(b)	No.	% ^(c)	No.	% ^(b)	No.	% ^(d)	No.	% ^(d)	No.	% ^(d)
I	1000	M	60	9 (4)	15.0	2 (2)	22.2	11	18.3	5	45.5	6	54.5	4	36.4
II	250	M	60	8 (3)	13.3	3 (1)	37.5	5	8.3	5	100.0	0	–	0	–
III	0 ^(e)	M	60	4	6.7	1	25.0	3	5.0	1	33.3	2	66.7	0	–

^(a) Between brackets the number of animals also bearing interstitial cell adenomas

^(b) Percentages refer to the total number of animals

^(c) Percentages refer to the total number of animals bearing interstitial cell hyperplasias

^(d) Percentages refer to the total number of animals bearing interstitial cell adenomas (composed of monomorphic basophile cells, and monomorphic/polymorphic eosinophile cells, or cells of both types)

^(e) Olive oil alone

Table 3 - CRC/RF project: long-term carcinogenicity bioassays on methyl-tertiary-butyl ether (MTBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 958). Results: haemolymphoreticular neoplasias among female rats: incidence and distribution by histocytotype

Group No.	Dose (mg/kg b.w. in olive oil)	Sex	No. of animals at start	Animals bearing haemolymphoreticular neoplasias							
				Total ^(a)		Lymphoblastic lymphoma ^(b)		Lymphoblastic leukaemia ^(b)		Lympho-immunoblastic lymphoma ^(b)	
				No.	%	No.	%	No.	%	No.	%
I	1000	F	60	12	20.0	3	25.0	1	8.3	8	66.7
II	250	F	60	7	11.7	0	–	1	14.3	6	85.7
III	0 ^(c) (control)	F	60	2	3.3	1	50.0	0	–	1	50.0

^(a) Percentages refer to the number of animals at start

^(b) Percentages refer to the total number of animals bearing haemolymphoreticular neoplasias

^(c) Olive oil alone

Table 4 - CRC/RF project: long-term carcinogenicity bioassays on methyl-tertiary-butyl ether (MTBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 958). Results: distribution of haemolymphoreticular neoplasias (HLRN) in female rats by site

Group No.	Dose (mg/kg b.w. in olive oil)	Sex	No. of animals at start	No. of HLRN	Distribution of haemolymphoreticular neoplasias by site ^(a)				
					Sites involved	Total No.	Lymphoblastic lymphoma No.	Lymphoblastic leukaemia No.	Lympho-immunoblastic lymphoma No.
I	1000	F	60	12	Nodes	9	4	1	4
					Spleen	4	2	1	1
					Liver	5	2	2	1
					Lung	11	3	1	7
					Other	3	2	1	0
II	250	F	60	7	Nodes	1	0	0	1
					Spleen	2	0	1	1
					Liver	1	0	1	0
					Lung	5	0	0	5
					Other	0	0	0	0
III	0 ^(b) (control)	F	60	2	Nodes	2	1	0	1
					Spleen	2	1	0	1
					Liver	1	1	0	0
					Lung	0	0	0	0
					Other	1	0	0	1

^(a) Haemolymphoreticular neoplasias may affect many different anatomical sites; in this table all localizations have been taken into account

^(b) Olive oil alone

Table 5 - CRC/RF project: long-term carcinogenicity bioassays on methyl-tertiary-butyl ether (MTBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 958). Results: haemolymphoreticular dysplasias among female rats

Group No.	Dose (mg/kg b.w. in olive oil)	Sex	No. of animals at start	Animals bearing haemolymphoreticular dysplasias ^(a) (lymphoimmunoblastic dysplasia)	
				No.	%
I	1000	F	60	12	20.0
II	250	F	60	16	26.7
III	0 ^(b) (control)	F	60	3	5.0

^(a) The dysplasias observed in animals bearing lymphomas and leukaemias are not included

^(b) Olive oil alone

Table 6 - Physical and chemical properties of ethyl-tertiary-butyl ether (ETBE)

Molecular weight	102
Density (g/l)	0.74
Water solubility (g/100 g water)	1.2
Octanol-water partition coefficient	NA
Octane number	112
Vapour pressure	
torr at 25°C	130
psi (RVP) at 100°F	4
blending RVP	4
Boiling point (°C)	72.70
Volume % gasoline for	
2% oxygen by weight	12.8
2.7% oxygen by weight	17
Odor detection threshold (ppm) ^(a)	0.013
Odor recognition threshold ^(a)	0.024
Test threshold ^(a)	0.047

^(a) ETBE = 99% purity

fluctuations in, the different types of tumour in control animals. After weaning, at 4-5 weeks of age, the experimental animals were identified by ear punch, randomized in order to have not more than one male and one female of each litter in the same group, and housed 5 per cage. The animals were 8 weeks old at the start of the experiment.

The plan of the experiment is shown in Table 7.

The compound was administered by gavage in extra virgin olive oil solution. The solutions were prepared weekly and maintained at 4°C. Every single test dose of ETBE was delivered in 1 ml daily solution, 4 days weekly (Monday and Tuesday, Thursday and Friday), for 104 weeks. An administration of the compound at the highest dose for 5-6 days weekly, would not have been tolerated

Table 7 - CRC/RF project: long-term carcinogenicity bioassays on ethyl-tertiary-butyl ether (ETBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 959). Results: plan of the experiment

Group	Dose (mg/kg b.w. in olive oil)	Age at start (weeks)	Animals	
			Sex	No.
I	1000	8	M	60
			F	60
			M+F	120
II	250	8	M	60
			F	60
			M+F	120
III	0 ^(a)	8	M	60
			F	60
			M+F	120

^(a) Olive oil alone

by the rats. Control animals were given 1 ml of extra virgin olive oil alone following the same calendar.

The animals were kept under observation until natural death, under highly standardized housing, diet and experimental conduct conditions, identical to those used in the CRC/RF Laboratories over the last 25 years. In experiments performed at the CRC/RF, the animals are habitually kept under observation for the life-span, in order to allow for development of all neoplastic potentialities. Mean daily drinking water and feed 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 for the first 13 weeks of the experiment, 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 137 weeks, with the death of the last animal at the age of 145 weeks (September 28th, 1995).

Upon death, the animals were submitted to systematic necropsy. Histopathology was routinely performed on all the macroscopically observed pathological lesions (with a margin of surrounding normal tissue) and on skin and subcutaneous tissue, the brain, pituitary gland, Zymbal glands, salivary glands, Harderian glands,

cranium (with oral and nasal cavities and external and internal ear ducts) (5 levels), tongue, thyroid and parathyroid, pharynx, larynx, thymus and mediastinal lymph nodes, trachea, lung and mainstem bronchi, heart, diaphragm, liver, spleen, pancreas, kidneys and adrenal glands, oesophagus, stomach (fore and glandular), intestine (4 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 preserved in 70% ethyl alcohol, except for the bones which were fixed in 10% formalin and then decalcified with 10% formaldehyde and 20% formic acid in water solution. The normal specimens were trimmed, following Standard Operating Procedures (SOP) at the CRC/RF Laboratories: i.e. parenchymal organs were dissected through the hilus to expose the widest surface, and hollow organs were sectioned across the greatest diameter(s). The pathological tissue was trimmed through the largest surface, including normal adjacent tissues. 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 tumours and any other lesion of oncological interest. All pathologists followed the same criteria of histopathological evaluation and classification.

Fig. 1 - Mean daily water consumption in male rats (BT 959).

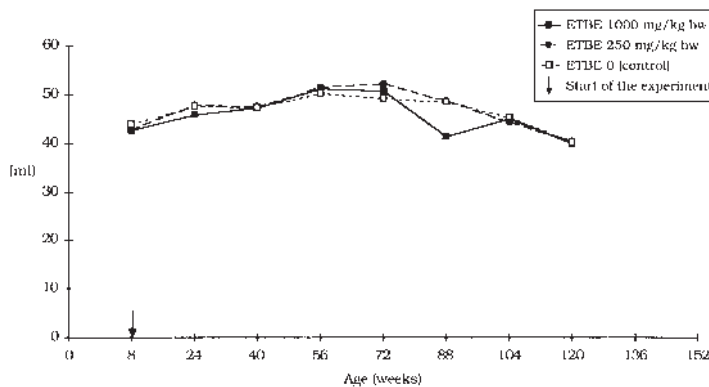
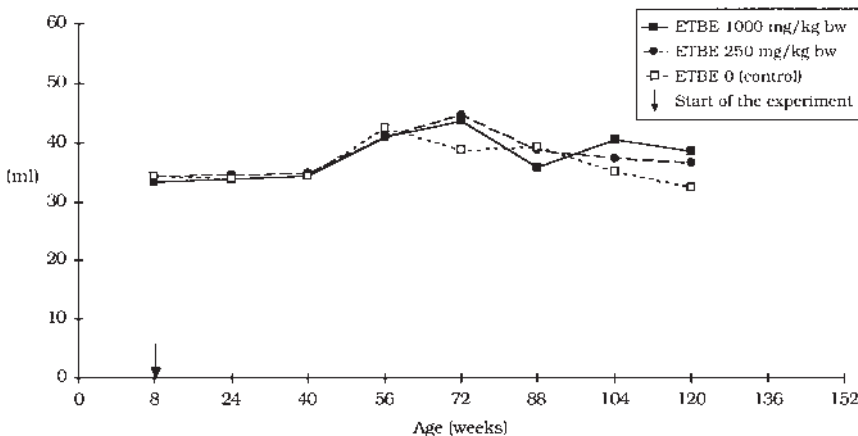


Fig. 2 - Mean daily water consumption in female rats (BT 959).



The experiment was conducted in conformity with the principles of Good Laboratory Practices (GLP).

Statistical analysis was performed using the χ^2 test, in order to evaluate the level of significance in tumour incidence differences between treated and control groups.

Results

No major differences were observed, among the groups, in mean daily water consumption (figs. 1 and 2), in mean daily feed consumption (figs. 3 and 4), in mean body weight (figs. 5 and 6).

There was a dose-correlated increase of the mortality rate in males, starting from the 40th week until the end of the experiment (fig. 7), and in females from the 40th to 88th week of the biophase (fig. 8).

The incidence of benign and malignant tumours by site and histotype, among male and female rats of the three experimental groups, is shown in Tables 8 and 9.

The incidence of total benign tumours is higher among male and female rats of the control group (Table 10). The decrease in the

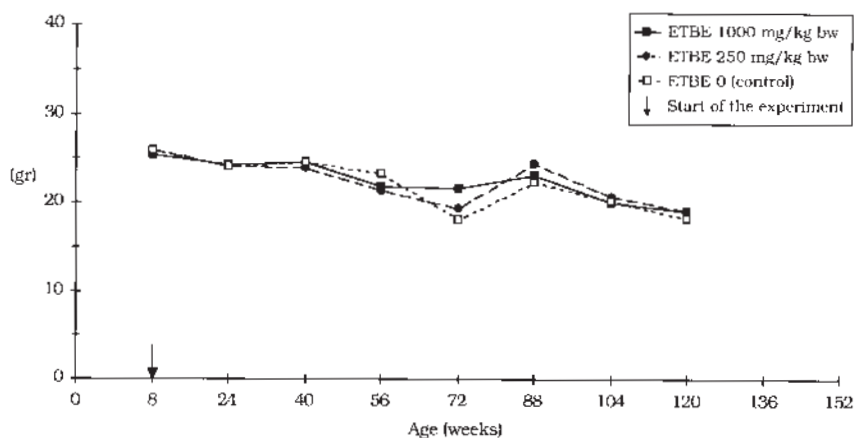


Fig. 3 - Mean daily feed consumption in male rats (BT 959).

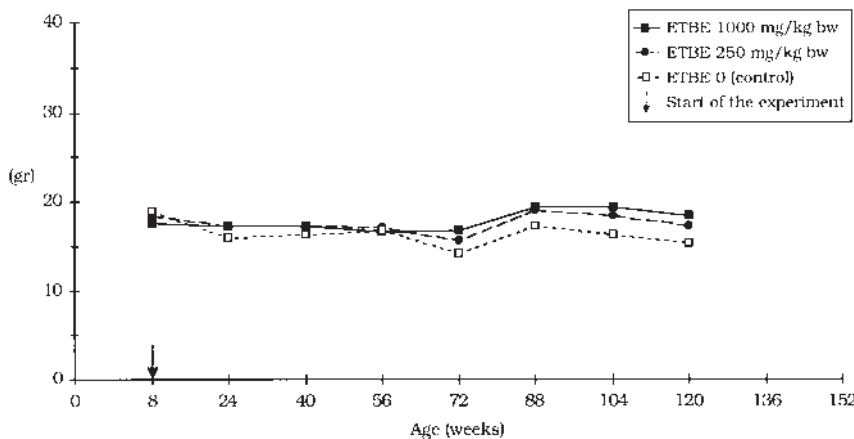


Fig. 4 - Mean daily feed consumption in female rats (BT 959).

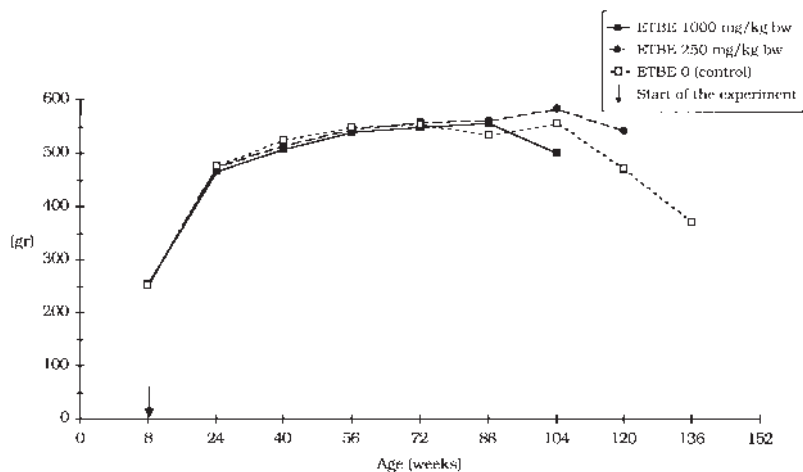


Fig. 5 - Mean body weight in male rats (BT 959).

Fig. 6 - Mean body weight in female rats (BT 959).

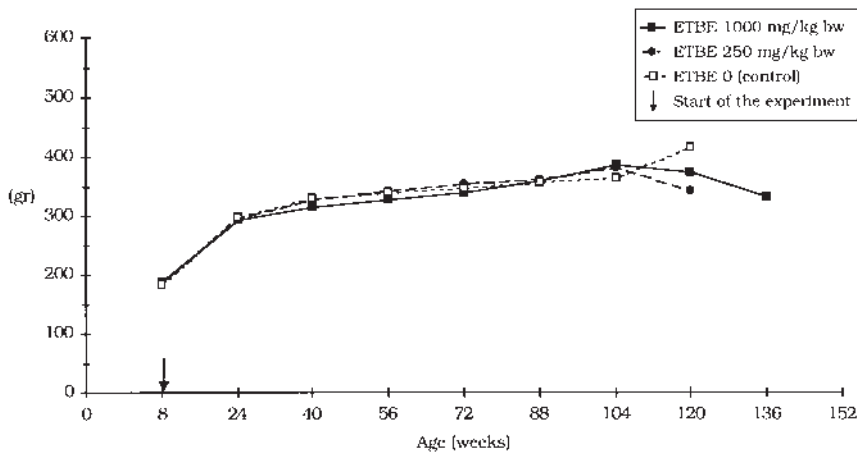


Fig. 7 - Survival of male rats (BT 959).

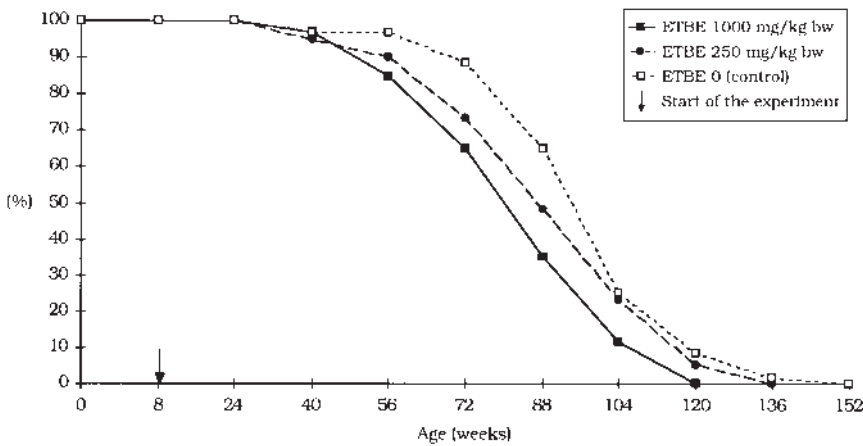
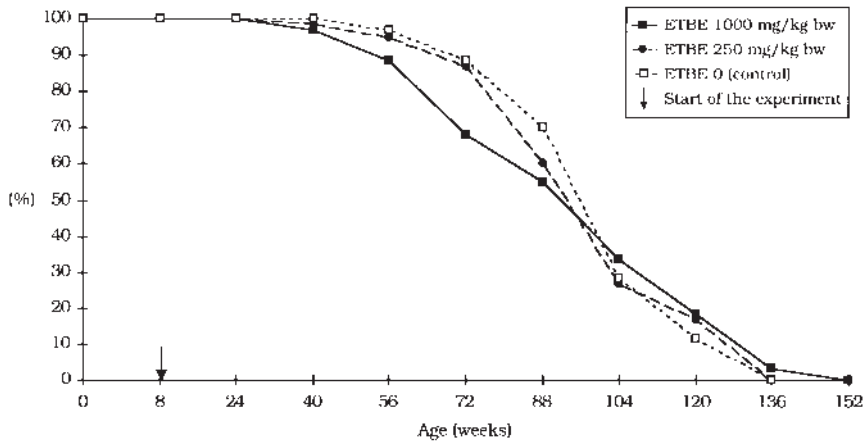


Fig. 8 - Survival of female rats (BT 959).



treated groups is ETBE dose-correlated. This effect must be put down to the shorter survival in the treated groups, especially the one treated with the higher dose.

The incidence of total malignant tumours, in spite of the higher mortality, is elevated in the treated groups, particularly among females, although with no dose-relationship (Table 11).

An increase in total pathologies of oncological interest of the mouth epithelium was observed in the animals of the two exposed groups, mainly among males (Table 12).

An increase in total oncologically relevant pathologies of the forestomach was found among males exposed to the lower dose of ETBE (Table 13). Of note in this group was the onset of an unusual number of squamous cell carcinomas of the forestomach, in both males and females.

An unusually high incidence of malignant tumours of the uterus, mainly sarcomas, was observed among females exposed to the lower dose (Table 14).

An increase in the incidence of haemolymphoreticular neoplasias,

Table 8 - CRC/RF project: long-term carcinogenicity bioassays on ethyl-tertiary-butyl ether (ETBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 959). Number and percentage of male Sprague-Dawley rats bearing various type of benign and malignant tumours ^(a)

Site	Histotype	Groups					
		I: 1000 mg/kg b.w.		II: 250 mg/kg b.w.		III: 0 ^(b) (control)	
		No.	%	No.	%	No.	%
Skin	Dermatofibroma	1	1.7	0	–	0	–
	Squamous cell carcinoma	0	–	0	–	1	1.7
Subcutaneous tissue	Fibroma	1	1.7	0	–	0	–
	Lipoma	0	–	0	–	1	1.7
	Liposarcoma	2	3.3	0	–	2	3.3
Mammary gland	Fibroma and fibroadenoma	5	8.3	2	3.3	5	8.3
	Fibrolipoma	0	–	0	–	4	6.7
	Lipoma	0	–	0	–	1	1.7
	Adenocarcinoma	1	1.7	0	–	2	3.3
	Liposarcoma	0	–	1	1.7	0	–
Ear ducts	Carcinoma	0	–	2	3.3	0	–
Nasal cavities	Olfactory neuroblastoma	1	1.7	0	–	0	–
Oral cavity, lips and tongue ^(c)	Acanthoma	2	3.3	0	–	1	1.7
	Carcinoma	1	1.7	0	–	0	–
Lung	Adenoma	0	–	0	–	1	1.7
Stomach – Forestomach ^(d)	Acanthoma	4	6.7	7	18.3	5	8.3
	Squamous cell carcinoma	0	–	3	5.0	0	–
	Leiomyosarcoma	1	1.7	0	–	0	–
Pancreas	Islet cell adenoma	2	3.3	3	5.0	1	1.7
	Exocrine adenoma	0	–	0	–	1	1.7
	Islet cell adenocarcinoma	0	–	0	–	2	3.3
Testes	Interstitial cell adenoma	0	–	1	1.7	0	–
Pituitary gland	Adenoma	23	38.3	29	48.3	28	46.7
Thyroid gland	C-cell adenoma	1	1.7	1	1.7	4	6.7
Adrenal glands	Cortical adenoma	0	–	7 (8)	11.7	2	3.3
	Pheochromocytoma	6 (7)	5.0	15 (21)	25.0	15 (17)	25.0
	Cortical adenocarcinoma	0	–	1	1.7	0	–
	Pheochromoblastoma	1	1.7	0	–	1	1.7
Central nervous system – Brain	Oligodendroglioma	0	–	0	–	1	1.7
Bones – Cranium – Other	Osteosarcoma	3	5.0	1	1.7	0	–
	Osteosarcoma	1	1.7	0	–	0	–
Soft tissues	Glomic tumour	0	–	0	–	1	1.7
	Mixoma	0	–	0	–	1	1.7
	Liposarcoma	0	–	0	–	1	1.7
Heart	Malignant Schwannoma	1	1.7	0	–	0	–
Spleen	Fibroangioma	0	–	1	1.7	0	–
Haemolymphoreticular tissues ^{(e)(f)}	Lymphomas and leukaemias	6	10.0	8	13.3	3	5.0

^(a) Between brackets the number of tumours (one animal can bear more than one tumour)^(b) Olive oil alone^(c) See table 12^(d) See table 13^(e) Including spleen^(f) See tables 15 and 16

Table 9 - CRC/RF project: long-term carcinogenicity bioassays on ethyl-tertiary-butyl ether (ETBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 959). Number and percentage of female Sprague-Dawley rats bearing various type of benign and malignant tumours ^(a)

Site	Histotype	Groups					
		I: 1000 mg/kg b.w.		II: 250 mg/kg b.w.		III: 0 ^(b) (control)	
		No.	%	No.	%	No.	%
Skin	Dermatofibroma	1	1.7	0	–	1	1.7
	Basal cell carcinoma	0	–	1	1.7	1	1.7
Subcutaneous tissue	Lipoma	0	–	1	1.7	0	–
Mammary gland	Fibroma and fibroadenoma	22 (30)	36.7	30 (39)	50.0	33 (59)	55.0
	Adenocarcinoma	4	6.7	4	6.7	3	5.0
	Liposarcoma	1	1.7	0	–	0	–
Zymbal glands	Carcinoma	1	1.7	1	1.7	0	–
Ear ducts	Carcinoma	1	1.7	2	3.3	1	1.7
Oral cavity, lips and tongue ^(c)	Acanthoma	3	5.0	2	3.3	1	1.7
	Carcinoma	1	1.7	2	3.3	0	–
Pleura	Mesothelioma	0	–	0	–	1	1.7
Stomach – Forestomach ^(d)	Acanthoma	6	10.0	3	5.0	5	8.3
	Carcinoma	0	–	3	5.0	0	–
– Glandular	Leiomyosarcoma	0	–	0	–	1	1.7
Pancreas	Exocrine adenoma	0	–	1	1.7	1	1.7
	Islet cell adenoma	1	1.7	1	1.7	0	–
Pelvis	Transitional cell carcinoma	0	–	1	1.7	0	–
Bladder	Adenomatous polyp	0	–	1	1.7	0	–
Ovaries	Fibroangioma	1	1.7	0	–	0	–
	Theca cell tumour	0	–	0	–	1	1.7
	Granulosa and theca cell tumour	0	–	1	1.7	0	–
Uterus ^(e)	Polyp	14	23.3	11	18.3	14	23.3
	Leiomyoma	2	3.3	0	–	0	–
	Squamous cell carcinoma	0	–	1	1.7	1	1.7
	Adenocarcinoma	0	–	1	1.7	0	–
	Leiomyosarcoma	0	–	2	3.3	1	1.7
	Malignant Schwannoma	0	–	2	3.3	0	–
Uterus and vagina	Malignant Schwannoma	2	3.3	4	6.7	0	–
Peritoneum	Angioma	0	–	1	1.7	0	–
Pituitary gland	Adenoma	22	36.7	24	40.0	24	40.0
Thyroid gland	C-cell adenoma	3	5.0	6	10.0	2	3.3
	Adenocarcinoma	1	1.7	0	–	0	–
Adrenal glands	Cortical adenoma	3 (4)	5.0	5	8.3	12 (13)	20.0
	Pheochromocytoma	26 (32)	43.3	27 (39)	45.0	29 (35)	48.3
	Cortical adenocarcinoma	1	1.7	0	–	0	–
	Pheochromoblastoma	1	1.7	0	–	0	–
Central nervous system – Brain	Oligodendroglioma	0	–	0	–	1	1.7
	– Meninges	Benign meningioma	1	1.7	0	–	0
Bones – Cranium	Osteosarcoma	4	6.7	3	5.0	2	3.3
Soft tissues	Lipoma	0	–	1	1.7	0	–
Spleen	Fibroangioma	1	1.7	0	–	0	–
Haemolymphoreticular tissues ^{(f) (g)}	Lymphomas and leukaemias	5	8.3	6	10.3	3	5.0

^(a) Between brackets the number of tumours (one animal can bear more than one tumour)^(b) Olive oil alone^(c) See table 12^(d) See table 13^(e) See table 14^(f) Including spleen^(g) See tables 15 and 17

Table 10 - CRC/RF project: long-term carcinogenicity bioassays on ethyl-tertiary-butyl ether (ETBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 959). Total benign tumours

Group No.	Dose (mg/kg b.w. in olive oil)	Animals		Benign tumours			
		Sex	No.	Animal bearing tumours		Tumours	
				N.	%	N.	Per 100 animals
I	1000	M	60	32	53.3	46	76.7
		F	60	49	81.7	121	201.7
		M+F	120	81	67.5	167	139.2
II	250	M	60	40	66.7	73	121.7
		F	60	53	88.3	136	226.7
		M+F	120	93	77.5	209	174.2
III	0 ^(a) (control)	M	60	40	66.7	75	125.0
		F	60	50	83.3	156	260.0
		M+F	120	90	75.0	231	192.5

^(a) Olive oil alone**Table 11** - CRC/RF project: long-term carcinogenicity bioassays on ethyl-tertiary-butyl ether (ETBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 959). Total malignant tumours

Group No.	Dose (mg/kg b.w. in olive oil)	Animals		Malignant tumours			
		Sex	No.	Animal bearing tumours		Tumours	
				N.	%	N.	Per 100 animals
I	1000	M	60	14	23.3	18	30.0
		F	60	19	31.6	22	36.7
		M+F	120	33	27.5	40	33.3
II	250	M	60	14	23.3	16	26.7
		F	60	21	35.0	33	55.0 ^(a)
		M+F	120	35	29.2	49	40.8
III	0 ^(b) (control)	M	60	11	18.3	13	21.6
		F	60	9	15.0	15	25.0
		M+F	120	20	16.6	28	23.3

^(a) Statistically significant ($p \leq 0.05$) using χ^2 test^(b) Olive oil alone**Table 12** - CRC/RF project: long-term carcinogenicity bioassays on ethyl-tertiary-butyl ether (ETBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 959). Pathologies of oncological interest of the mouth epithelium (oral cavity, tongue and lips)

Group No.	Dose (mg/kg b.w. in olive oil)	Animals		Acanthomas		Sq. cell dysplasias		Sq. cell dysplasias borderline with in situ carcinoma		Sq. cell carcinomas		Total	
		Sex	No.										
				No.	%	N.	%	No.	%	No.	%	N.	%
I	1000	M	60	2	3.3	11	18.3	1	1.7	1	1.7	15	25.0 ^(a)
		F	60	3	5.0	12	20.0	2	3.3	1	1.7	18	30.0
		M+F	120	5	4.2	23	19.2	3	2.0	2	1.7	33	25.5
II	250	M	60	0	–	14	23.3	0	–	0	–	14	23.3
		F	60	2	3.3	11	18.3	1	1.7	2	3.3	16	26.7
		M+F	120	2	1.7	25	20.8	1	0.8	2	1.7	30	25.0
III	0 ^(b) (control)	M	60	1	1.7	5	8.3	0	–	0	–	6	10.0
		F	60	1	1.7	11	18.3	2	3.3	0	–	14	23.3
		M+F	120	2	1.7	16	13.3	2	1.7	0	–	20	16.7

^(a) Statistically significant ($p \leq 0.05$) using χ^2 test^(b) Olive oil alone

mainly lymphoimmunoblastic lymphoma, was found in males and females of the two treated groups, in spite of the shorter survival (Table 15). The three cases of myeloid leukaemias in animals treated at the lower dose deserve attention. The distribution of all these neoplasias by histotype and site is presented in Tables

16 and 17. The lung is the most frequent site of lymphoimmunoblastic lymphoma.

No increase in testicular interstitial cell adenomas was detected in the groups exposed to ETBE.

The fact that the increase in oncological pathology is evident, on-

Table 13 - CRC/RF project: long-term carcinogenicity bioassays on ethyl-tertiary-butyl ether (ETBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 959). Pathologies of oncological interest of the forestomach

Group No.	Dose (mg/kg b.w. in olive oil)	Animals		Acanthomas		Sq. cell dysplasias		Sq. cell carcinomas		Total	
		Sex	No.	No.	%	N.	%	No.	%	No.	%
I	1000	M	60	4	6.7	9	15.0	0	–	13	21.7
		F	60	6	10.0	5	8.3	0	–	11	18.3
		M+F	120	10	8.3	14	11.7	0	–	24	20.0
II	250	M	60	7	11.7	14	23.3	3	5.0	24	40.0
		F	60	3	5.0	4	6.7	3	5.0	10	16.7
		M+F	120	10	8.3	18	15.0	6	5.0	34	28.3
III	0 ^(a) (control)	M	60	5	8.3	8	13.3	0	–	13	21.7
		F	60	5	8.3	7	11.7	0	–	12	20.0
		M+F	120	10	8.3	15	12.5	0	–	25	20.8

^(a) Olive oil alone

Table 14 - CRC/RF project: long-term carcinogenicity bioassays on ethyl-tertiary-butyl ether (ETBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 959). Malignant tumours of the uterus

Group No.	Dose (mg/kg b.w. in olive oil)	No. of animals at start	Animals bearing malignant tumours of the uterus					
			Carcinomas		Sarcomas		Total	
			N.	%	N.	%	N.	%
I	1000	60	0	–	2 ^(a)	3.3	2	3.3
II	250	60	2	3.3	8 ^(b)	13.3	10	16.7 ^(c)
III	0 ^(d) (control)	60	1	1.7	1	1.7	2	3.3

^(a) 2 malignant Schwannomas of the uterus-vagina

^(b) 4 malignant Schwannomas of the uterus-vagina

^(c) Statistically significant ($p \leq 0.05$) using χ^2 test

^(d) Olive oil alone

Table 15 - CRC/RF project: long-term carcinogenicity bioassays on ethyl-tertiary-butyl ether (ETBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 959). Haemolymphoreticular neoplasias and their distribution by histocytotype

Group No.	Dose (mg/kg b.w. in olive oil)	Animals		Animals bearing haemolymphoreticular neoplasias											
		Sex	No.	Total ^(a)		Lympho-blastic lymphoma ^(b)		Lympho-cytic lymphoma ^(b)		Lymphoimmu-noblastic lymphoma ^(b)		Histiocytic sarcoma ^(b)		Myeloid leukaemia ^(b)	
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I	1000	M	60	6	10.0	0	–	0	–	5	83.3	1	16.7	0	–
		F	60	5	8.3	0	–	1	20.0	2	40.0	2	40.0	0	–
		M+F	120	11	9.2	0	–	1	9.1	7	63.6	3	27.3	0	–
II	250	M	60	8	13.3	1	12.5	0	–	4	50.0	1	12.5	2	33.3
		F	60	6	10.0	1	16.7	1	16.7	3	50.0	0	–	1	16.7
		M+F	120	14	11.6	2	14.3	1	7.1	7	50.0	1	7.1	3	21.4
III	0 ^(c) (control)	M	60	3	5.0	0	–	0	–	2	66.7	1	33.3	0	–
		F	60	3	5.0	0	–	0	–	1	33.3	2	66.7	0	–
		M+F	120	6	5.0	0	–	0	–	3	50.0	3	50.0	0	–

^(a) Percentages refer to the number of animals at start

^(b) Percentages refer to the total number of animals bearing haemolymphoreticular neoplasias

^(c) Olive oil alone

Table 16 - CRC/RF project: long-term carcinogenicity bioassays on ethyl-tertiary-butyl ether (ETBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 959). Distribution of haemolymphoreticular neoplasias (HLRN) in male rats by site

Group No.	Dose (mg/kg b.w. in olive oil)	Sex	No. of animals at start	No. of HLRN	Sites involved	Distribution of haemolymphoreticular neoplasias by site ^(a)					
						Total	Lymphoblastic lymphoma	Lymphocytic lymphoma	Lymphoimmunoblastic lymphoma	Histiocytic sarcoma	Myeloid leukaemia
						No.	No.	No.	No.	No.	No.
I	1000	M	60	6	Nodes	2	0	0	1	1	0
					Spleen	1	0	0	0	1	0
					Liver	1	0	0	0	1	0
					Lung	6	0	0	5	1	0
					Other	2	0	0	1	1	0
II	250	M	60	8	Nodes	6	1	0	2	1	2
					Spleen	3	0	0	0	1	2
					Liver	3	0	0	0	1	2
					Lung	7	1	0	4	0	2
					Other	3	0	0	1	0	2
III	0 ^(b)	M	60	3	Nodes	2	0	0	1	1	0
					Spleen	0	0	0	0	0	0
					Liver	1	0	0	0	1	0
					Lung	1	0	0	1	0	0
					Other	1	0	0	1	0	0

^(a) The haemolymphoreticular neoplasias may affect many different anatomical sites; in this table all localizations have been taken into account

^(b) Olive oil alone

Table 17 - CRC/RF project: long-term carcinogenicity bioassays on ethyl-tertiary-butyl ether (ETBE), administered by stomach tube to male (M) and female (F) Sprague-Dawley rats (Experiment BT 959). Distribution of haemolymphoreticular neoplasias (HLRN) in female rats by site

Group No.	Dose (mg/kg b.w. in olive oil)	Sex	No. of animals at start	No. of HLRN	Sites involved	Distribution of haemolymphoreticular neoplasias by site ^(a)					
						Total	Lymphoblastic lymphoma	Lymphocytic lymphoma	Lymphoimmunoblastic lymphoma	Histiocytic sarcoma	Myeloid leukaemia
						No.	No.	No.	No.	No.	No.
I	1000	F	60	5	Nodes	4	0	1	2	1	0
					Spleen	2	0	0	0	2	0
					Liver	2	0	0	0	2	0
					Lung	2	0	0	1	1	0
					Other	2	0	1	0	1	0
II	250	F	60	6	Nodes	6	1	1	3	0	1
					Spleen	2	0	0	1	0	1
					Liver	2	0	0	1	0	1
					Lung	3	0	0	2	0	1
					Other	3	0	1	1	0	1
III	0 ^(b)	F	60	3	Nodes	1	0	0	0	1	0
					Spleen	1	0	0	0	1	0
					Liver	2	0	0	0	2	0
					Lung	2	0	0	1	1	0
					Other	1	0	0	0	1	0

^(a) The haemolymphoreticular neoplasias may affect many different anatomical sites; in this table all localizations have been taken into account

^(b) Olive oil alone

ly or mainly, in animals treated at the low dose, may be explained by the early mortality in the group exposed to the higher dose of ETBE.

Discussion and conclusion

Our experimental study on the carcinogenicity of ETBE has several limitations: it considers only two dose levels of exposure, it

has only been performed in one animal system and both test doses caused a shortening of survival.

In spite of these limitations, the results of the study show that ETBE causes an increase in total malignant tumours and an increase in oncological pathologies of the mouth epithelium and forestomach, of malignant tumours of the uterus (mainly sarcomas), and of haemolymphoreticular neoplasias.

The effect of ETBE in causing an increase in haemolymphoretic-

ular neoplasias, mainly lymphoimmunoblastic lymphomas, is similar to that of MTBE tested by us in the same animal model and with the same experimental procedures.

The experiment here presented may be considered preliminary, but its results already indicate that ETBE cannot be considered safe.

The problems raised by fuels, and more specifically by gasoline, are multiple and enormous, since they cover: exploitation of crude oil resources, use of renewable fuels, economic aspects, environmental pollution and human health. The answers to such problems cannot rely upon sectorial proposals favouring one of these aspects – usually economic – without adequate wide-ranging scientific information. Such information necessarily extends to environmental and public health data. It is alarming that in the world of fuels the data are too scanty for any sound decisions to be reached.

What we do not need at present is such reasoning as: “since MTBE is risky, let us try ETBE; since ETBE may not be safe, let us try with another oxygenate, since oxygenates... let us try with...”, without having any solid scientific basis to support the proposals. What we do need, instead, is to step up research.

References

- American Petroleum Institute (API): Alcohols and ethers: a technical assessment of their application as fuels and fuel components. 2nd ed. API publication 4261. American Petroleum Institute, Washington, DC, 1988.
- Belpoggi F., Soffritti M., Filippini F., *et al.*: Results of long-term experimental studies on the carcinogenicity of methyl-tert-butyl ether. In: E. Bingham, and D. Rall: Preventive strategies for living in a chemical world, 77-95. Annals of New York Academy of Science, Vol. 837, New York, 1997.
- Belpoggi F., Soffritti M., and Maltoni C.: Methyl-tertiary-butyl ether (MTBE) – a gasoline additive – causes testicular and lymphohaematopoietic cancers in rats. *Toxicol. Ind. Health*, **11**, 119-149, 1995.
- Belpoggi F., Soffritti M., and Maltoni C.: Pathological characterization of testicular tumours and lymphomas-leukaemias, and of their precursors observed in Sprague-Dawley rats exposed to methyl-tertiary-butyl ether (MTBE). *Eur. J. Oncol.*, **3**, 201-206, 1998.
- Burleigh-Flayer H.D., Chun J.S., and Kintish W.J.: Methyl-tertiary-butyl ether: vapor inhalation oncogenicity study in CD-1 mice. BRRC report 91N0013A. Union Carbide, Bushy Run Research Center, Export, PA, 1992.
- Chun J.S., Burleigh-Flayer H.D., and Kintish W.J.: Methyl-tertiary-butyl ether: vapor inhalation oncogenicity study in Fisher-344 rats. BRRC report 91N0013B. Union Carbide, Bushy Run Research Center, Export, PA, 1992.
- United States Environmental Protection Agency (US EPA): Guidance on estimating motor vehicle emission reductions from the use of alternative fuels blends. EPA technical report no. EPA-AA-TSS-PA-87-4, Office of Mobile Sources, Ann Arbor, MI, 1988.