

# Results of Long-Term Carcinogenicity Bioassays on *Tert*-Amyl-Methyl-Ether (TAME) and Di-Isopropyl-Ether (DIPE) in Rats

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**ABSTRACT:** *Tert*-amyl-methyl ether (TAME) was administered by gavage in extra virgin olive oil solution at concentrations of 750, 250, or 0 mg/kg bw to groups of 100 male and 100 female Sprague-Dawley rats 8 weeks old at the start of the experiment. Di-isopropyl ether (DIPE) was administered in the same manner at the doses of 1000, 250, or 0 mg/kg body weight to groups of 100 male and 100 female Sprague-Dawley rats. TAME and DIPE were each delivered in 1-mL solution 4 days a week for 78 weeks. Control animals received 1 mL of extra virgin olive oil without TAME or DIPE. At the end of the treatment period, all animals were kept under observation until spontaneous death. Under these test conditions, TAME and DIPE were found to be potential carcinogenic agents for various organs and tissues.

**KEYWORDS:** oxygenated gasoline additives; *tert*-amyl-methyl-ether; di-isopropyl-ether; carcinogenicity; long-term bioassay; rat

## INTRODUCTION

The gasoline oxygenated additives proposed at present include ethers, such as methyl-*tert*-butyl ether (MTBE), ethyl-*tert*-butyl ether (ETBE), *tert*-amyl-methyl ether (TAME), di-isopropyl ether (DIPE), and alcohols such as methyl alcohol, ethyl alcohol, or *tert*-butyl alcohol (TBA). Adding oxygenates to gasoline increases its oxygen content and allegedly reduces emissions of CO and possibly some air toxics, such as ozone-forming hydrocarbons and benzene. However, these oxygenates may increase toxic aldehydes, such as formaldehyde or acetaldehyde. When the use of gasoline-oxygenated additives, particularly MTBE, began in the 1970s, there were no precise data on their impact on the environment and health. No scientific information was available on the carcinogenicity of these compounds.

Only in the middle 1990s were the results of two different experiments on carcinogenicity of MTBE made available. In one study, conducted at the Cancer Research

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Center of the Ramazzini Foundation (CRC/RF), MTBE was shown to cause an increase in lymphomas/leukemias (mainly due to lymphoimmunoblastic lymphomas) in female Sprague-Dawley rats and an increase in interstitial cell adenomas of the testis in male rats.<sup>1-3</sup> Another study, sponsored by producers and users of MTBE, found that MTBE caused an increase in hepatocellular adenoma in female CD-1 mice and an increase in renal tubular adenomas and carcinomas in male Fisher 344 rats.<sup>4,5</sup>

In recent years, great concern has arisen regarding the use of MTBE because of groundwater contamination associated with gasoline spills and leaks from underground storage tanks<sup>6</sup> and complaints of people exposed to MTBE from such sources, complaining of unpleasant odor, headaches, and burning of the eyes and throat.<sup>7</sup> Because of MTBE's toxic effects, carcinogenic potential, air pollution, and contamination of the water supply, the petroleum industry has had to propose other oxygenated additives as alternative oxygenated gasoline additives.

In the 1980s, in the context of our research program on the carcinogenicity of fuels, a systematic and integrated project of experimental carcinogenicity bioassay was started on various gasoline additives, namely, the oxygenated additives methyl alcohol, ethyl alcohol, MTBE, ETBE, TAME, DIPE, and the isoparaffin, 2,2,4-trimethyl pentane (TMP). The experiments were performed on Sprague-Dawley rats from the CRC/RF colony on which there is abundant information regarding expected pathology from historical controls. The final results of the experiments on MTBE and ETBE have already been published,<sup>1-3,8</sup> and those on methyl alcohol and ethyl alcohol are reported in this volume. This report outlines the final results of the carcinogenicity bioassays on TAME and DIPE.

*Tert*-amyl-methyl ether (TAME) is a colorless, flammable liquid. TAME (C<sub>6</sub>H<sub>14</sub>O) has a molecular weight of 102.18. Di-isopropyl ether (DIPE) is a colorless, flammable liquid with a sharp, sweet, ether-like odor. DIPE (C<sub>6</sub>H<sub>14</sub>O) has a molecular weight of 102.18.

TAME is manufactured from isoamylene and methanol feedstocks. The principal source of isoamylene is the C5-olefin stream from a crude oil-refining process called fluid catalytic cracking. TAME manufacturing provides a refinery with a way to reduce the gasoline vapor pressure, reduce the light olefin content of gasoline, and create a high octane gasoline-blending component. However, similar benefits could also be obtained by using the C5-olefins in the refinery's hydrocarbon alkylation process.<sup>9</sup> DIPE is commercially prepared by the action of sulfuric acid on isopropyl alcohol and also obtained as a by-product in the production of isopropyl alcohol from the propylene fraction of cracked gasoline.<sup>10</sup> The world production capacity of DIPE is unknown but likely to be small. In the United States, it is a permitted additive under U.S. Federal reformulated gasoline regulations.<sup>11</sup>

The information on toxicity, mutagenicity, and carcinogenicity on TAME is extremely limited.<sup>12</sup>

## MATERIAL AND METHODS

TAME and DIPE were supplied by SIGMA-ALDRICH, Division of SAF Bulk Chemicals, Milan, Italy, and their purity was higher than 97% and 98%, respectively. The extra virgin oil used as a carrier was provided by Oliaria Toscana (the same oil used in the CRC/RF laboratory for 25 years).

**TABLE 1. Long-term carcinogenicity bioassays on *tert*-amyl-methyl ether (TAME) administered by gavage to male (M) and female (F) Sprague-Dawley rats**

NUMBER AND PERCENTAGE OF SPRAGUE-DAWLEY RATS BEARING VARIOUS TYPES OF BENIGN AND MALIGNANT TUMORS <sup>a</sup>												
Site Histotype	Groups											
	I: 750 mg/kg b. w.				II: 250 mg/kg b. w.				III: 0 <sup>b</sup> (control)			
	Male		Female		Male		Female		Male		Female	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<b>Skin</b>												
Dermatofibroma	0	-	0	-	1	1.0	0	-	1	1.0	0	-
Sebaceous adenoma	0	-	0	-	0	-	0	-	0	-	1	1.0
Squamous cell carcinoma	0	-	0	-	0	-	0	-	1	1.0	0	-
Basocellular carcinoma	0	-	0	-	0	-	1	1.0	0	-	0	-
<b>Subcutaneous tissue</b>												
Fibrolipoma	2	2.0	0	-	0	-	0	-	0	-	0	-
<b>Mammary glands</b>												
Fibroma and fibroadenoma	2	2.0	42(61)	42.0	3	3.0	52(70)	52.0	8(10)	8.0	34(42)	34.0
Lipoma and fibrolipoma	3	3.0	1	1.0	3	3.0	1	1.0	3	3.0	0	-
Fibroangioma	1	1.0	0	-	0	-	0	-	0	-	0	-
Adenocarcinoma	0	-	7(9)	7.0	0	-	14(18)	14.0	0	-	10(15)	10.0
Fibrosarcoma	0	-	0	-	0	-	0	-	0	-	1	1.0
Liposarcoma	0	-	0	-	1	1.0	0	-	1	1.0	2	2.0
Angiosarcoma	1	1.0	0	-	0	-	0	-	0	-	0	-
<b>Zymbal glands</b>												
Acanthoma	0	-	0	-	1	1.0	0	-	0	-	0	-
Sebaceous adenoma	0	-	0	-	0	-	0	-	1	1.0	1	1.0
Carcinoma	1	1.0	0	-	1	1.0	1	1.0	2(3)	2.0	2	2.0
<b>Ear ducts</b>												
Sebaceous adenoma	0	-	0	-	0	-	0	-	1	1.0	0	-
Carcinoma	5	5.0	4	4.0	4	4.0	4(5)	4.0	1	1.0	2	2.0
<b>Nasal cavities</b>												
Carcinoma	0	-	1	1.0	0	-	1	1.0	2	2.0	0	-
<b>Oral cavity, tongue and lips</b>												
Carcinoma	0	-	0	-	0	-	0	-	1	1.0	1	1.0
<b>-Tooth</b>												
Odontoma	0	-	0	-	0	-	0	-	1	1.0	0	-
<b>Pharynx</b>												
Carcinoma	0	-	0	-	0	-	0	-	1	1.0	0	-

— Continued

TABLE 1. *Continued*

Site Histotype	Groups													
	I. 750 mg/kg b. w.				II. 250 mg/kg b. w.				III. 0 <sup>b</sup> (control)					
	Male		Female		Male		Female		Male		Female			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
Lung														
Angioma	1	1.0	0	-	0	-	0	-	0	-	0	-	0	-
Leiomyosarcoma	1	1.0	0	-	1	1.0	0	-	0	-	0	-	0	-
Stomach														
- Fore stomach														
Acanthoma	3	3.0	1	1.0	0	-	2	2.0	2	2.0	1	1.0	1	1.0
- Glandular stomach														
Leiomyosarcoma	0	-	0	-	0	-	1	1.0	0	-	0	-	0	-
Intestine														
Glandular polyp	0	-	1	1.0	0	-	0	-	0	-	0	-	0	-
Adenomatous polyp	0	-	0	-	0	-	0	-	0	-	1	1.0	1	1.0
Adenocarcinoma	0	-	0	-	1	1.0	0	-	0	-	1	1.0	1	1.0
Leiomyosarcoma	0	-	0	-	1	1.0	1	1.0	0	-	0	-	0	-
Salivary glands														
Adenoma	0	-	1	1.0	0	-	0	-	0	-	0	-	0	-
Liver														
Cholangioma	0	-	0	-	0	-	0	-	1	1.0	0	-	0	-
Hepatocarcinoma	1	1.0	0	-	0	-	0	-	0	-	0	-	0	-
Pancreas														
Exocrine adenoma	0	-	0	-	1	1.0	2	2.0	0	-	2	2.0	2	2.0
Islet cell adenoma	2	2.0	0	-	2	2.0	0	-	5	5.0	2	2.0	2	2.0
Islet cell carcinoma	0	-	0	-	0	-	1	1.0	0	-	0	-	0	-
Kidneys														
Neproblastoma	1	1.0	0	-	0	-	0	-	2	2.0	0	-	0	-
Adenocarcinoma	0	-	0	-	2	2.0	0	-	0	-	0	-	0	-
Pelvis and ureters														
Transitional cell papilloma	0	-	0	-	0	-	1	1.0	0	-	0	-	0	-
Testes														
Interstitial cell adenoma	4	4.0	0	-	3	3.0	0	-	0	-	0	-	0	-
Ovaries														
Cystadenoma			0	-			1	1.0			1	1.0		
Granulosa cell tumor			0	-			1	1.0			0	-		
Adenocarcinoma			0	-			1	1.0			0	-		

— *Continued*

TABLE 1. *Continued*

Site Histotype	Groups											
	I: 750 mg/kg b. w.			II: 250 mg/kg b. w.			III: 0 <sup>b</sup> (control)					
	No.	%	%	No.	%	%	No.	%	%	No.	%	%
Uterus												
Polyp	13		13.0	14		14.0				10		10.0
Leiomyoma	6		6.0	1		1.0				1		1.0
Squamous cell carcinoma	0		-	1		1.0				0		-
Adenocarcinoma	1		1.0	2		2.0				3		3.0
Leiomyosarcoma	2		2.0	0		-				0		-
Angiosarcoma	2		2.0	0		-				0		-
Uterus & Vagina												
Malignant Schwannoma	1		1.0	1		1.0				3		3.0
Vagina												
Fibroma	0		-	0		-				2		2.0
Benign Schwannoma	1		1.0	0		-				0		-
Squamous cell carcinoma	0		-	0		-				1		1.0
Malignant Schwannoma	1		1.0	0		-				1		1.0
Peritoneum												
Mesothelioma	0		-	1		1.0	0		-	3		3.0
Pituitary gland												
Adenoma	38		38.0	26		26.0	27		27.0	25		25.0
Adenocarcinoma	0		-	0		-	2		2.0	0		-
Thyroid gland												
Follicular adenoma	0		-	0		-	0		-	0		-
C-cell adenoma	1		1.0	3		3.0	0		-	2		2.0
Parathyroid glands												
Adenoma	0		-	0		-	0		-	1		1.0
Adrenal glands												
Cortical adenoma	2		2.0	5		5.0	0		-	5		5.0
Phaeochromocytoma	7(8)		7.0	9(10)		9.0	6(8)		6.0	6(8)		6.0
Cortical adenocarcinoma	0		-	2		2.0	0		-	5(6)		5.0
Phaeochromoblastoma	0		-	0		-	1		1.0	1(2)		1.0
Sympathoblastoma	0		-	0		-	1		1.0	0		-

— *Continued*

TABLE 1. *Continued*

Site Histotype	Groups													
	I: 750 mg/kg b. w.				II: 250 mg/kg b. w.				III: 0 <sup>b</sup> (control)					
	Male		Female		Male		Female		Male		Female			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
Central nervous system														
- Brain														
Multiform glioblastoma	0	-	1	1.0	0	-	0	-	0	0	0	-	0	-
Oligodendroglioma	3	3.0	2	2.0	4	4.0	2	2.0	0	0	2	2.0	2	2.0
- Meninges														
Benign meningioma	0	-	0	-	0	-	0	-	0	0	1	1.0	1	1.0
Peripheral nervous system														
- Major peripheral nerves														
Malignant Schwannoma	0	-	0	-	1	1.0	0	-	2	2.0	0	-	0	-
Bones														
- Head														
Osteosarcoma	7	7.0	2	2.0	1	1.0	4	4.0	2	2.0	7	7.0	7	7.0
- Other sites														
Osteosarcoma	0	-	0	-	0	-	1	1.0	1	1.0	1	1.0	1	1.0
Soft tissues														
Histiocytoma	0	-	0	-	0	-	1	1.0	0	0	0	-	0	-
Leiomyosarcoma	0	-	0	-	0	-	1	1.0	0	0	0	-	0	-
Liposarcoma	0	-	1	1.0	0	-	0	-	0	0	0	-	0	-
Heart														
Myxoma	0	-	0	-	0	-	3	3.0	0	0	0	-	0	-
Malignant Schwannoma	1	1.0	0	-	2	2.0	0	-	0	0	0	-	0	-
Spleen														
Fibrosarcoma	0	-	0	-	0	-	0	-	0	0	1	1.0	1	1.0
Angiosarcoma	0	-	0	-	1	1.0	0	-	0	0	0	-	0	-
Pericytosarcoma	0	-	0	-	0	-	0	-	0	0	0	-	1	1.0
Hemolymphoreticular tissues <sup>c, d</sup>														
Lymphomas and leukemias	21	21.0	27(28)	27.0	7	7.0	14	14.0	17	17.0	7	7.0	7	7.0

<sup>a</sup> Between brackets the number of tumors (one animal can bear more than one tumor)

<sup>b</sup> Olive oil alone

<sup>c</sup> Including spleen

<sup>d</sup> See table 3

**TABLE 2. Long-term carcinogenicity bioassays on *tert*-amyl-methyl ether (TAME) administered by gavage to male (M) and female (F) Sprague-Dawley rats**

TOTAL MALIGNANT TUMORS							
Group No.	Daily dose (mg/kg b.w. in olive oil)	Animals		Malignant tumors			
		Sex	No.	Tumor-bearing animals		Tumors	
				No.	%	No.	Per 100 animals
I	750	M	100	37	37.0	42	42.0
		F	100	44	44.0	58	58.0
		M+F	200	81	40.5	100	50.0
II	250	M	100	24	24.0	31	31.0
		F	100	46	46.0	67	67.0
		M+F	200	70	35.0	98	49.0
III	0 <sup>a</sup>	M	100	30	30.0	34	34.0
		F	100	37	37.0	58	58.0
		M+F	200	67	33.5	92	46.0

<sup>a</sup> Olive oil alone

During experiments, compounds TAME and DIPE were stored at 4°C. TAME was administered by gavage in 1 mL extra virgin olive oil solution at concentrations of 750, 250, or 0 mg/kg bw to groups of 100 male and 100 female Sprague-Dawley rats 8 weeks old at the start of the experiment. TAME was administered daily, 4 days weekly, for 78 weeks. The animals were then maintained under control conditions until spontaneous death. Control animals received 1 mL of extra virgin olive oil without TAME. The experiment began in February 1995 and ended after 135 weeks of treatment with the death of the last animal at 143 weeks of age. Testing of DIPE was concurrent with that of TAME, and the experimental protocols were identical, apart from the higher dose level of DIPE (1000 mg/kg body weight). The DIPE experiment ended after 163 weeks of treatment with the death of the last animal at 171 weeks of age. Both experiments were performed according to Good Laboratory Practices (GLP) and Standard Operating Procedure (SOP) of the CRC/RF.

The experimental conditions, protocol, and histopathology are described in detail elsewhere in this volume.<sup>13</sup> Multiple tumors of different type and site, of different type in the same site, of the same type in bilateral organs, of the same type in the skin, subcutaneous tissue, and mammary glands, or at distant sites of diffuse tissue (i.e., bones, skeletal muscle, etc.) were plotted as single/independent tumors. Multiple tumors of the same type in the same tissue and organ, including the bilateral organs, were plotted only once. Statistical analysis was performed using the  $\chi^2$  test to evaluate differences in tumor incidence between treated and control groups. The Cochran Armitage test was used to evaluate dose-response relationships.

**TABLE 3. Long-term carcinogenicity bioassays on *tert*-amyl-methyl ether (TAME) administered by gavage to male (M) and female (F) Sprague-Dawley rats**

HEMOLYMPHORETICULAR NEOPLASIAS AND THEIR DISTRIBUTION BY HISTOCYTOTYPE																	
Group No.	Daily dose (mg/kg b.w. in olive oil)	Animals		Animals with hemolymphoreticular neoplasias													
		Sex	No.	Total <sup>a</sup>		Lymphoblastic lymphoma <sup>b</sup>		Lymphoblastic leukemia		Lymphocytic lymphoma <sup>b</sup>		Lymphoimmuno-blastic lymphoma <sup>b</sup>		Histiocytic sarcoma/monocytic leukemia		Myeloid leukemia <sup>b</sup>	
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I	750	M	100	21	21.0	0	-	0	-	0	-	17	81.0	4	19.0	0	-
		F	100	27 <sup>c</sup>	27.0 <sup>***♦♦</sup>	1	3.8	0	-	1	3.7	21	77.8	5	18.6	0	-
		M+F	200	48 <sup>c</sup>	24.0	1	2.1	0	-	1	2.1	38	79.2	9	18.8	0	-
II	250	M	100	7	7.0	0	-	0	-	0	-	6	85.7	1	14.3	0	-
		F	100	14	14.0 <sup>♦♦</sup>	2	14.3	0	-	2	14.3	6	42.9	4	28.6	0	-
		M+F	200	21	10.5	2	9.5	0	-	2	9.5	12	57.1	5	23.8	0	-
III	0 <sup>d</sup>	M	100	17	17.0	0	-	0	-	0	-	13	76.5	3	17.6	1	5.9
		F	100	7	7.0	3	42.9	0	-	0	-	3	42.9	1	14.3	0	-
		M+F	200	24	12.0	3	12.5	0	-	0	-	16	66.7	4	16.7	1	4.2

<sup>a</sup> Percentages refer to the number of animals at start

<sup>b</sup> Percentages refer to the number of animals bearing hemolymphoreticular neoplasias

<sup>c</sup> One animal bore a lymphoblastic lymphoma and histiocytic sarcoma

<sup>d</sup> Olive oil alone

<sup>\*\*\*</sup> p<0.01 using  $\chi^2$  test

<sup>♦♦</sup> p<0.05 using Cochrane-Armitage test for dose-response relationship

**TABLE 4. Long-term carcinogenicity bioassays on di-isopropyl (DIPE)) administered by gavage to male (M) and female (F) Sprague-Dawley rats**

NUMBER AND PERCENTAGE OF SPRAGUE-DAWLEY RATS BEARING VARIOUS TYPES OF BENIGN AND MALIGNANT TUMORS <sup>a</sup>												
Site	Groups											
	I: 1,000 mg/kg b. w.				II: 250 mg/kg b. w.				III: 0 <sup>b</sup> (control)			
	Male		Female		Male		Female		Male		Female	
Histotype	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<b>Skin</b>												
Acanthoma	0	-	0	-	1	1.0	1	1.0	0	-	0	-
Cheratoacanthoma	1	1.0	0	-	0	-	0	-	0	-	0	-
Dermatofibroma	1	1.0	0	-	1	1.0	0	-	1	1.0	0	-
Squamous cell carcinoma	0	-	0	-	0	-	0	-	1	1.0	0	-
Sebaceous adenoma	0	-	0	-	0	-	0	-	0	-	1	1.0
<b>Subcutaneous tissue</b>												
Fibroma	1	1.0	0	-	2	2.0	0	-	0	-	0	-
Angiosarcoma	0	-	0	-	1	1.0	0	-	0	-	0	-
Fibrosarcoma	0	-	0	-	0	-	1	1.0	0	-	0	-
Liposarcoma	0	-	0	-	1	1.0	0	-	0	-	0	-
<b>Mammary glands</b>												
Fibroma and fibroadenoma	4	4.0	42(53)	42.0	4(5)	4.0	44(60)	44.0	8(10)	8.0	34(42)	34.0
Myxoma	0	-	0	-	1	1.0	0	-	0	-	0	-
Lipoma and fibrolipoma	2	2.0	0	-	5	5.0	0	-	3	3.0	0	-
Adenocarcinoma	0	-	7(8)	7.0	0	-	12(15)	12.0	0	-	10(15)	10.0
Fibrosarcoma	0	-	1	1.0	0	-	0	-	0	-	1	1.0
Liposarcoma	0	-	0	-	1	1.0	0	-	1	1.0	2	2.0
Angiosarcoma	0	-	0	-	0	-	1	1.0	0	-	0	-
<b>Zymbal glands</b>												
Sebaceous adenoma	1	1.0	0	-	0	-	0	-	1	1.0	1	1.0
Carcinoma	0	-	1	1.0	1	1.0	3	3.0	2(3)	2.0	2	2.0
<b>Ear ducts</b>												
Sebaceous adenoma	0	-	0	-	0	-	0	-	1	1.0	0	-
Carcinoma	4	4.0	4	4.0	9(11)	9.0	3(4)	3.0	1	1.0	2	2.0

— Continued

TABLE 4. *Continued*

Site	Groups												
	I: 1,000 mg/kg b. w.				II: 250 mg/kg b. w.				III: 0 <sup>c</sup> (control)				
	Male		Female		Male		Female		Male		Female		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Nasal cavities													
Carcinoma	0	-	0	-	0	-	0	-	2	2.0	0	-	-
Oral cavity, tongue and lips													
Carcinoma	0	-	1	1.0	0	-	0	-	1	1.0	1	1.0	1.0
Tooth													
Adenomatina	0	-	1	1.0	0	-	0	-	0	-	0	-	0
Odontoma	0	-	0	-	0	-	0	-	1	1.0	0	-	-
Pharynx													
Carcinoma	0	-	0	-	0	-	0	-	1	1.0	0	-	-
Lung													
Adenoma	0	-	0	-	1	1.0	0	-	0	-	0	-	0
Fibroangioma	0	-	0	-	0	-	1	1.0	0	-	0	-	0
Stomach													
- Forestomach													
Acanthoma	0	-	2	2.0	4	4.0	2	2.0	2	2.0	1	1.0	1.0
Lipoma	1	1.0	0	-	0	-	0	-	0	-	0	-	0
- Glandular stomach													
Adenocarcinoma	0	-	1	1.0	0	-	0	-	0	-	0	-	0
Intestine													
Adenomatous polyp	0	-	0	-	0	-	0	-	0	-	1	1.0	1.0
Leiomyoma	0	-	1	1.0	0	-	0	-	0	-	0	-	0
Adenocarcinoma	0	-	0	-	0	-	0	-	0	-	0	-	0
Leiomyosarcoma	1	1.0	0	-	0	-	0	-	0	-	0	-	0
Salivary glands													
Adenocarcinoma	0	-	0	-	1	1.0	0	-	0	-	0	-	0

— *Continued*

TABLE 4. *Continued*

Site	NUMBER AND PERCENTAGE OF SPRAGUE-DAWLEY RATS BEARING VARIOUS TYPES OF BENIGN AND MALIGNANT TUMORS <sup>a</sup>											
	I: 1,000 mg/kg b. w.				II: 250 mg/kg b. w.				III: 0 <sup>b</sup> (control)			
	Male		Female		Male		Female		Male		Female	
Histotype	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Liver	0	-	1	1.0	0	-	0	-	1	1.0	0	-
Pancreas												
Exocrine adenoma	1	1.0	0	-	0	-	3	3.0	0	-	2	2.0
Islet cell adenoma	3	3.0	2	2.0	9	9.0	2	2.0	5	5.0	2	2.0
Exocrine adenocarcinoma	0	-	0	-	1	1.0	1	1.0	0	-	0	-
Islet cell carcinoma	0	-	0	-	1	1.0	1	1.0	0	-	0	-
Kidneys												
Nephroblastoma	0	-	0	-	0	-	0	-	2	2.0	0	-
Prostate												
Adenoma	0	-			3	3.0			0	-		
Seminal vesicles												
Adenoma	0	-			1	1.0			0	-		
Testes												
Interstitial cell adenoma	2	2.0			2	2.0			0	-		
Ovaries												
Cystadenoma			1	1.0			3(4)	3.0			1	1.0
Granulosa cell tumor			1	1.0			0	-			0	-
Sertoli cell tumor			0	-			3(4)	3.0			0	-
Malignant granulosa cell tumor			0	-			3	3.0			0	-
Uterus												
Polyp			15	15.0			8	8.0			10	10.0
Leiomyoma			4	4.0			2	2.0			1	1.0
Fibromioma			1	1.0			1	1.0			0	-
Adenocarcinoma			3	3.0			3	3.0			3	3.0
Malignant Schwannoma			1	1.0			0	-			0	-

— *Continued*

TABLE 4. *Continued*

Site	NUMBER AND PERCENTAGE OF SPRAGUE-DAWLEY RATS BEARING VARIOUS TYPES OF BENIGN AND MALIGNANT TUMORS <sup>a</sup>															
	I: 1,000 mg/kg b. w.						II: 250 mg/kg b. w.						III: 0 <sup>b</sup> (control)			
	Male			Female			Male			Female			Male		Female	
	No.	%		No.	%		No.	%		No.	%		No.	%	No.	%
Uterus & Vagina																
Malignant Schwannoma				5	5.0		8	8.0							3	3.0
Vagina																
Fibroma				0	-		0	-							2	2.0
Squamous cell carcinoma				0	-		0	-							1	1.0
Malignant Schwannoma				1	1.0		2	2.0							1	1.0
Peritoneum																
Mesothelioma	0	-		2	2.0		0	-		2	2.0		0	-	1	1.0
Pituitary gland																
Adenoma	43	43.0		24	24.0		50	50.0		38	38.0		44	44.0	18	18.0
Adenocarcinoma	1	1.0		1	1.0		0	-		1	1.0		0	-	0	-
Thyroid gland																
Follicular adenoma	0	-		0	-		0	-		2	2.0		0	-	1	1.0
C-cell adenoma	2	2.0		5	5.0		2	2.0		1	1.0		1	1.0	4	4.0
Follicular adenocarcinoma	0	-		0	-		0	-		2	2.0		0	-	0	-
C-cell carcinoma	0	-		1	1.0		3	3.0		0	-		0	-	0	-
Parathyroid glands																
Adenoma	0	-		1	1.0		0	-		0	-		0	-	1	1.0
Adrenal glands																
Cortical adenoma	0	-		1	1.0		2	2.0		3	3.0		1	1.0	8(9)	8.0
Pheochromocytoma	12	12.0		19(25)	19.0		15(18)	15.0		15(17)	15.0		6(8)	6.0	9(10)	9.0
Cortical adenocarcinoma	0	-		4	4.0		1	1.0		4	4.0		0	-	2	2.0
Pheochromoblastoma	2	2.0		0	-		0	-		2	2.0		0	-	5	5.0

— *Continued*

TABLE 4. *Continued*

NUMBER AND PERCENTAGE OF SPRAGUE-DAWLEY RATS BEARING VARIOUS TYPES OF BENIGN AND MALIGNANT TUMORS <sup>a</sup>													
Site	Histotype	Groups											
		I: 1,000 mg/kg b. w.				II: 250 mg/kg b. w.				III: 0 <sup>b</sup> (control)			
		Male		Female		Male		Female		Male		Female	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
Central nervous system													
- Brain													
	Oligodendroglioma	3	3.0	2	2.0	4	4.0	3	3.0	0	-	2	2.0
	Glioblastoma	0	-	0	-	0	-	1	1.0	0	-	0	-
- Meninges													
	Benign meningioma	1	1.0	0	-	2	2.0	0	-	0	-	1	1.0
Peripheral nervous system													
- Major peripheral nerves													
	Malignant Schwannoma	0	-	1	1.0	2	2.0	0	-	2	2.0	0	-
- Ganglia													
	Ganglioneuroma	0	-	0	-	1	1.0	0	-	0	-	0	-
Skeletal muscle													
	Rhabdomyosarcoma	0	-	1	1.0	0	-	0	-	0	-	0	-
Bones													
- Head													
	Osteoma	0	-	0	-	0	-	1	1.0	0	-	0	-
	Osteosarcoma	6	6.0	4	4.0	2	2.0	3	3.0	2	2.0	7	7.0
- Other sites													
	Osteosarcoma	1	1.0	0	-	0	-	1	1.0	1	1.0	1	1.0
Soft tissues													
	Fibroangioma	0	-	0	-	1	1.0	0	-	0	-	0	-
	Sarcoma	0	-	0	-	0	-	1	1.0	0	-	0	-
Heart													
	Myxoma	0	-	2	2.0	0	-	1	1.0	0	-	0	-
Thymus													
	Malignant thymoma <sup>c</sup>	0	-	0	-	1	1.0	0	-	0	-	0	-
Spleen													
	Fibroangioma	0	-	1	1.0	0	-	0	-	0	-	1	1.0
	Pericytosaoma	0	-	0	-	0	-	0	-	0	-	1	1.0
Subcutaneous lymph nodes													
	Angioma	0	-	1	1.0	0	-	0	-	0	-	0	-
Hemolymphoreticular tissues <sup>d e</sup>													
	Lymphomas and leukemias	31	31.0	41	41.0	27	27.0	28	28.0	17	17.0	7	7.0

<sup>a</sup>Between brackets the number of tumors (one animal can bear more than one tumor). <sup>b</sup>Olive oil alone. <sup>c</sup>In 96% of cases the tumor itself is composed of a mixture in varying proportions of epithelial cells and lymphocytes. In the remaining 4%, only epithelial cells are present. We consider that a tumor composed exclusively of lymphocytes should not be classified as a thymoma but as a lymphoma involving the thymus. <sup>d</sup>Including thymus, spleen and subcutaneous lymph nodes. <sup>e</sup>See TABLES 6.

**TABLE 5. Long-term carcinogenicity bioassays on di-isopropyl ether (DIPE) administered by gavage to male (M) and female (F) Sprague-Dawley rats**

TOTAL MALIGNANT TUMORS								
Group No.	Daily dose (mg/kg b.w. in olive oil)	Animals		Malignant tumors				
		Sex	No.	Tumor-bearing animals		Tumors		
				No.	%	No.	Per 100 animals	
I	1,000	M	100	38	<b>38.0</b>	***♦♦	49	<b>49.0</b>
		F	100	57	<b>57.0</b>		85	<b>85.0</b>
		M+F	200	95	<b>47.5</b>		134	<b>67.0</b>
II	250	M	100	46	<b>46.0</b> *	***♦♦	58	<b>58.0</b> **
		F	100	55	<b>55.0</b>		90	<b>90.0</b> **
		M+F	200	101	<b>50.5</b>		148	<b>74.0</b>
III	0 <sup>a</sup>	M	100	30	30.0		34	34.0
		F	100	37	37.0		58	58.0
		M+F	200	67	33.5		92	46.0

<sup>a</sup> Olive oil alone

\* p< 0.05 using  $\chi^2$  test

\*\* p< 0.01 using  $\chi^2$  test

♦♦ p<0.01 using Cochran-Armitage test for dose-response relationship

**TABLE 6. Long-term carcinogenicity bioassays on di-isopropyl ether (DIPE) administered by gavage to male (M) and female (F) Sprague-Dawley rats**

<u>HEMOLYMPHORETICULAR NEOPLASIAS AND THEIR DISTRIBUTION BY HISTOCYTOTYPE</u>																	
Group No.	Daily dose (mg/kg b.w. in olive oil)	Animals		Animals with hemolymphoreticular neoplasias													
				Total <sup>a</sup>		Lymphoblastic lymphoma <sup>b</sup>		Lymphoblastic leukemia		Lymphocytic lymphoma <sup>b</sup>		Lymphoimmunoblastic lymphoma <sup>b</sup>		Histiocytic sarcoma/monocytic leukemia <sup>b</sup>		Myeloid leukemia <sup>b</sup>	
		Sex	No.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I	1,000	M	100	31	<b>31.0</b> <sup>**♦</sup>	2	6.5	0	-	0	-	22	71.0	7	22.6	0	-
		F	100	41 <sup>c</sup>	<b>41.0</b> <sup>***♦♦</sup>	1	2.4	0	-	1	2.4	33	80.5	8	19.5	0	-
		M+F	200	72 <sup>c</sup>	<b>36.0</b>	3	4.2	0	-	1	1.4	55	76.4	15	20.8	0	-
II	250	M	100	27	<b>27.0</b> <sup>♦</sup>	2	7.4	0	-	0	-	20	74.1	5	18.5	0	-
		F	100	28	<b>28.0</b> <sup>***♦♦</sup>	1	3.6	0	-	6	21.4	15	53.6	5	17.9	1	3.6
		M+F	200	55	<b>27.5</b>	3	5.5	0	-	6	10.9	35	63.6	10	18.2	1	1.8
III	0 <sup>d</sup>	M	100	17	17.0	0	-	0	-	0	-	13	76.5	3	17.6	1	5.9
		F	100	7	7.0	3	42.9	0	-	0	-	3	42.9	1	14.3	0	-
		M+F	200	24	12.0	3	12.5	0	-	0	-	16	66.7	4	16.7	1	4.2

<sup>a</sup> Percentages refer to the number of animals at start

<sup>b</sup> Percentages refer to the total number of animals bearing hemolymphoreticular neoplasias

<sup>c</sup> Two animals bore a lymphoimmunoblastic lymphoma and histiocytic sarcoma

<sup>d</sup> Olive oil alone

\* p < 0.05 using  $\chi^2$  test

\*\* p < 0.01 using  $\chi^2$  test

♦ p < 0.05 using Cochran-Armitage test for dose-response relationship

♦♦ p < 0.01 using Cochran-Armitage test for dose-response relationship

## RESULTS

No significant differences were observed in daily water or feed consumption, body weight, behavior, or treatment-related nononcological pathological changes between TAME- or DIPE-treated and control animals. In the period between the 40th and 104th week of age, a decrease in survival was observed in TAME-treated males compared to controls. A decrease in survival was observed in DIPE-treated males versus controls in the period between the 56th and 88th week of age.

The occurrence of benign and malignant tumors in TAME-treated and control animals is shown in TABLE 1. Significant findings in TAME-treated and control animals are summarized as follows: (1) no differences were observed in the total number of malignant tumors (TABLE 2); (2) increases in some sporadic malignant tumors of the gastrointestinal tract occurred in males and females treated with the lower dose compared to the control group; (3) an increased incidence of ear duct carcinomas was noted in treated males and females compared to control animals; (4) an increase in the incidence of interstitial cell adenomas of the testis was observed in treated animals; (5) an increase in the incidence of glial malignant tumors of the brain was noted in males treated at both doses; and (6) an increase in the incidence of hemolymphoreticular neoplasias in males treated at the highest dose and in females treated at both doses (TABLE 3).

The occurrence of benign and malignant tumors in DIPE-treated and control animals is shown in TABLE 4. Significant findings in DIPE-treated and control animals are summarized as follows: (1) an increase in total malignant tumors was observed in males and females of both treated groups (TABLE 5); (2) an increase in the incidence of carcinomas of the ear duct occurred in males treated at 1,000 and 250 mg/kg bw ( $P < 0.05$ ) and in treated females; (3) the onset of some interstitial cell adenomas of the testis was noted in the treated group; (4) a slight increase in malignant sarcomas of the uterus and vagina was observed in the treated group; (5) an increase in the incidence of glial malignant tumors of the brain occurred in male- and female-treated animals; and (6) an increase of hemolymphoreticular neoplasias was observed in males and females of the treated groups (TABLE 6).

## CONCLUSIONS

TAME and DIPE were demonstrated to be potential carcinogenic agents for various organs and tissues.

The present experiments have some limitations: only two doses were studied, and the number of animals per group, although higher than usually foreseen for long-term bioassays, may not have been sufficient to completely show the carcinogenic potency of the compound tested.

If TAME and DIPE are expected to have increased commercial use, our data show that further carcinogenicity studies are essential before their introduction. Future animal studies should include a wider range of doses, a larger number of animals, and at least two animal models.

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