

Further Evidence of Benzene Carcinogenicity

Results on Wistar Rats and Swiss Mice Treated by Ingestion

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INTRODUCTION

Benzene is one of the most widely produced chemical compounds, and it has a wide variety of uses.

This compound has been known for more than half a century to cause leukemias in humans. The first demonstration that benzene is an experimental carcinogen was provided on Sprague-Dawley rats in 1977 by Maltoni and Scarnato, with an experiment performed at the Bentivoglio (BT) Experimental Unit of the Bologna Institute of Oncology.¹

In the same laboratory it was then shown that benzene is a multipotential strong carcinogen which causes a variety of tumors, some of which are of rare spontaneous occurrence, in different organs, when administered both by inhalation and ingestion.^{2,3}

The aim of this report is to present the results of long-term carcinogenicity bioassays with benzene given by ingestion to Wistar rats and Swiss mice.

The project was started in 1983 and the biophase was terminated in 1985.

BENZENE

Benzene (C₆H₆) has a molecular weight of 78.1 and its structural formula is



Data on the production, uses, and occurrence of the compound are given in TABLES 1, 2, and 3. The findings in the literature on carcinogenicity and other relevant effects are presented in TABLE 4.

TABLE 1. Benzene: Production

<i>Methods of Production</i>
Benzene is produced commercially from coal tar and mainly from petroleum. The various petroleum refinery techniques used in the production of benzene are: • catalytic reforming • hydrodealkylation • pyrolysis of gasoline • toluene disproportionation • dealkylation of higher alkyl aromatics
<i>World Production (1982)</i>
32 million tons per year (total global annual cycle, including benzene in fossil fuels)
<i>Geographical Areas of Production</i>
United States, Western Europe, Japan, and many other countries

TABLE 2. Benzene: Uses

<i>Major Uses</i>
As a component of gasoline, and as a chemical intermediate (for the production of ethylbenzene, cyclohexane, maleic anhydride, detergent alkylate, aniline, dichlorobenzenes, etc.)
<i>Other Uses</i>
As a solvent for paints and rubber; in the production of rubber cement; and in the manufacture of artificial leather
<i>Miscellaneous Applications</i>
It was used in the past in medicine for the treatment of hemoblastomas, and in veterinary medicine for disinfecting wounds

TABLE 3. Benzene: Occurrence

<i>Natural Sources</i>
It is believed that plants and animal matter release benzene to the environment; burning of wood and organic materials
<i>Workplaces</i>
Where benzene or products containing benzene are produced, used, stocked and distributed
<i>Air</i>
Of the 4 million tons estimated to be lost in the environment, the major part, given the high volatility of the compound, is emitted in the atmosphere. ¹ Benzene has been found in the air of • towns • general ambient • uncontaminated areas
<i>Water</i>
• drinking water
<i>Food and Drink</i>
• fruits, fish, vegetables, nuts, dairy products, beverages, eggs, cooked chicken and heat-treated or canned beef

TABLE 4. Benzene: Major Data on Carcinogenicity and Other Relevant Correlated Biological Effects

(1) <u>Toxicity</u>
(A) <i>Humans</i> :
(a) toxic effects on bone marrow (pancytopenia)
(B) <i>Animals</i> :
(a) toxic effects on bone marrow
(b) leukopenia in rats ²
(2) <u>Carcinogenicity studies</u>
(A) <i>Humans</i> :
(a) leukemias (mainly myeloblastic), and other hemolymphoreticular neoplastic diseases
(B) <i>Rat: by inhalation and by ingestion</i> : (a) in Sprague-Dawley rats, an increased incidence was observed of total malignant tumors; Zymbal gland carcinomas; oral and nasal cavities carcinomas; skin carcinomas; acanthomas, dysplasias and carcinomas of the forestomach; hepatomas; liver angiosarcomas; malignant mammary tumors; and hemolymphoreticular neoplasias ^{2,3}
(C) <i>Mouse: by inhalation</i> :
(a) in C57B1 mice, thymic lymphomas ⁷
(3) <u>Genetic effects</u> :
(A) Genotoxic in human and animal cells

LONG-TERM CARCINOGENICITY BIOASSAYS PERFORMED AT THE BENTIVOGLIO (BT) EXPERIMENTAL UNIT OF THE BOLOGNA INSTITUTE OF ONCOLOGY

Materials, Methods, Plan, and Conduct of the Experiments

Benzene was administered by ingestion (stomach **tube**) to Wistar rats **and** Swiss mice. Details on the test material and test animals are given in **TABLE 5**.

Glass syringes with a stainless steel needle having a round tip were used for the gavage.

The plan of the experiments is shown in **TABLES 6 and 7**. Details on the conduct of the experiments are presented in **TABLE 8**. Systematic and standardized histopathologic examinations were performed on each animal on the subcutaneous lymph nodes, brain and cerebellum, pituitary, Zymbal glands, interscapular

TABLE 5. Benzene: Test Compound and Test Animals

<u>Test Compound</u>	
(A) <i>Supplier</i> :	Montedison, Italy
(B) <i>Purity</i> :	
Benzene	99.93%
Paraffin	0.06%
Toluene	0.01%
<u>Test Animals</u>	
Male and female Wistar rats, 7 weeks old at the start of the experiment	
Male and female Swiss mice, 7 weeks old at the start of the experiment	
The animals were of the breed currently used in the BT Experimental Unit for many years	

TABLE 6. Benzene: Plan of the Experiment: Ingestion (Stomach Tube, 104 Weeks) in Wistar Rats (Exp. BT 907)

Group No.	Dose (mg/kg)	Animals	
		Sex	No. at Start
I	500	M	40
		F	40
		M + F	80
II	Olive oil (Control)	M	40
		F	40
		M + F	80
Total		M	80
		F	80
		M - F	160

TABLE 7. Benzene: Plan of the Experiment: Ingestion (Stomach Tube, 78 Weeks) in Swiss Mice (Exp. BT 908)

Group No.	Dose (mg/kg)	Animals	
		Sex	No. at Start
I	500	M	40
		F	40
		M + F	80
II	Olive oil (Control)	M	40
		F	40
		M + F	80
Total		M	80
		F	80
		M + F	160

TABLE 8. Benzene: Conduct of the Experiments

- The animals were exposed by ingestion (stomach tube) in olive oil once daily, 4-5 days weekly, for 104 weeks (rats) and for 78 weeks (mice).
- All the animals were kept under observation until spontaneous death.
- The *status* and behavior of the animals were examined 3 times daily.
- The animals were submitted to clinical examination for gross changes every 2 weeks.
- The animals were weighed every 2 weeks during treatment, and then every 8 weeks.
- Full necropsy was performed on all the animals.
- The housing and the diet of the animals were the same highly standardized ones adopted in the BT Experimental Unit during the last 15 years.

brown fat, salivary glands, Harderian glands, oral and nasal cavities (seven sections of the head), tongue, pharynx, thymus and mediastinal lymph nodes, lungs, diaphragm, liver, kidneys, adrenals, spleen, pancreas, esophagus, mesenteric lymph nodes, stomach, various segments of the intestine, bladder, uterus, gonads, and any other organs with pathologic lesions.

Results

Survival

The mortality was higher in benzene-treated male and female Wistar rats.

No relevant effects on the survival of treated Swiss mice compared with controls were observed.

Body Weight

Benzene treatment affects the body weight of both rats and mice, particularly of males.

Carcinogenicity

Rats. The most frequently expected tumors in the rats of the strain used, on the basis of the literature and of the historic controls of the BT Experimental Unit, are mammary tumors (benign and malignant), leukemias, pheochromocytomas, and pheochromoblastomas. Moreover, a variety of other miscellaneous tumors were also observed.^{6,7}

The data on the incidence of the total number of tumors and of the most frequent ones are given in TABLES 9 and 10. Benzene increased the incidence of total malignant tumors (TABLE 9). The higher relative incidence of total (benign and malignant) tumors in control groups is due to an enhanced number of benign neoplasias, as a consequence of the longer survival time of the animals treated with oil and of the decrease in body weight in treated animals, which affects the

TABLE 9. Benzene: Total Tumors in Rats Treated by Ingestion (Stomach Tube) (Exp. BT 907)

Group No.	Dose (mg/kg)	Animals		Percentage of Animals with Tumors		No. of Malignant Tumors per 100 Animals
		Sex	No. at Start	TBMT ^a	MT ^b	
I	500	M	40	57.5	47.5	60.0
		F	40	67.5	52.5	70.0
		M + F	80	62.5	50.0	65.0
II	Olive oil (Control)	M	40	75.0	20.0	25.0
		F	40	85.0	25.0	30.0
		M + F	80	80.0	22.5	27.5

^a Total benign and malignant tumors.

^b Malignant tumors.

TABLE 10. Benzene: Mammary Tumors, Leukemias, Pheochromocytomas, and Pheochromoblastomas in Rats Treated by Inhalation (Cumulative Tumor Incidence by RT 007)

Group No.	Dose (mg/kg)	Animals		Percentage in Animals with Tumors				
		Sex	No. at Start	Mammary Tumors		Leukemias	Pheochromocytomas	Pheochromoblastomas
				BMT ^a	MT ^b			
I	500	M	40	2.5	—	5.0	2.5	—
		F	40	42.5	5.0	10.0	—	—
II	Olive oil (Control)	M + F	80	22.5	2.5	7.5	1.2	—
		M	40	10.0	—	2.5	5.0	—
		F	40	55.0	7.5	7.5	5.0	—
		M + F	80	27.5	3.7	5.0	5.0	—

^a Benign and malignant tumors.

^b Malignant tumors.

^c The term "leukemia" includes a variety of hemolymphoreticular neoplastic diseases at different sites.

TABLE 11. Benzene: Carcinomas of the Zymbal Gland, Oral Cavity, and Nasal Cavities in Rats Treated by Ingestion (Stomach Tube) (Exp. BT 907)

Group No.	Dose (mg/kg)	Animals		Percentage of Animals with Tumors		
		Sex	No. at Start	Zymbal Gland Carcinomas	Oral Cavity Carcinomas	Nasal Cavity Carcinomas
I	500	M	40	17.5	5.0	5.0
		F	40	15.0	10.0	2.5
II	Olive oil (Control)	M + F	80	16.2	7.5	3.7
		M	40	—	—	—
		F	40	—	—	—
		M + F	80	—	—	—

onset of mammary tumors. In benzene-treated rats there was no increase in the incidence of the most frequent expected tumors, i.e., mammary tumors, leukemias, pheochromocytomas, and pheochromoblastomas. The higher incidence of malignant tumors in the benzene-treated animals is mainly due to the onset of carcinomas of the Zymbal gland, oral cavity, and nasal cavities (**TABLE 11**). The carcinomas of the Zymbal glands (**FIGS. 1 and 2**) and the oral cavity (**FIGS. 3 and 4**) are of squamous type; the ones of the nasal cavities are indifferiated (**FIG. 5**).

Mice. The most frequently expected tumors in the mice of the strain used, on the basis of the literature and of the historic controls of the BT Experimental Unit,

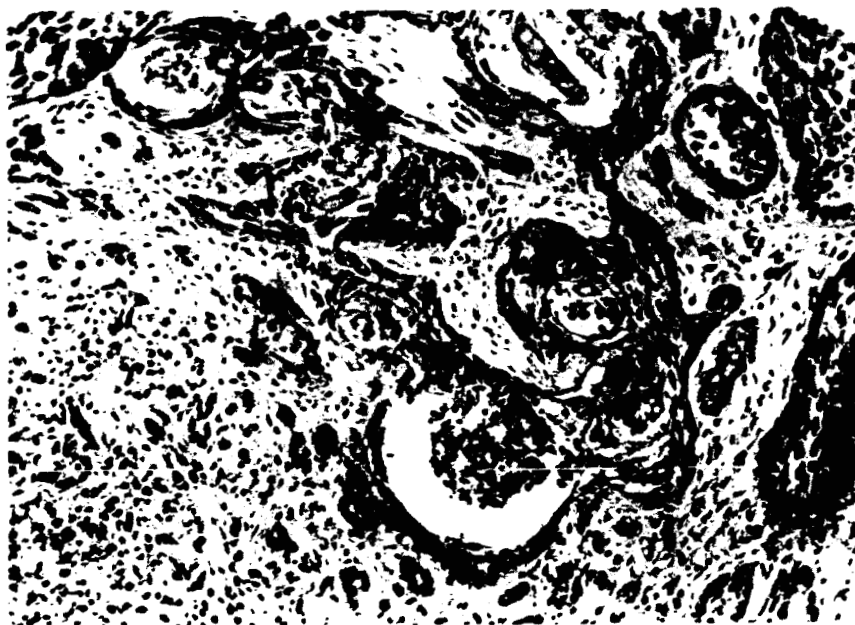
**FIGURE 1.** Zymbal gland squamous cell carcinoma in a male Wistar rat exposed to benzene by ingestion. (Hematoxylin and eosin stain. Original magnification $\times 200$; reduced by 15%.)



FIGURE 2. Lung metastasis of the Zymbal gland carcinoma shown in **FIGURE 1**. (Hematoxylin and eosin stain. Original magnification $\times 200$; reduced by 1%.)

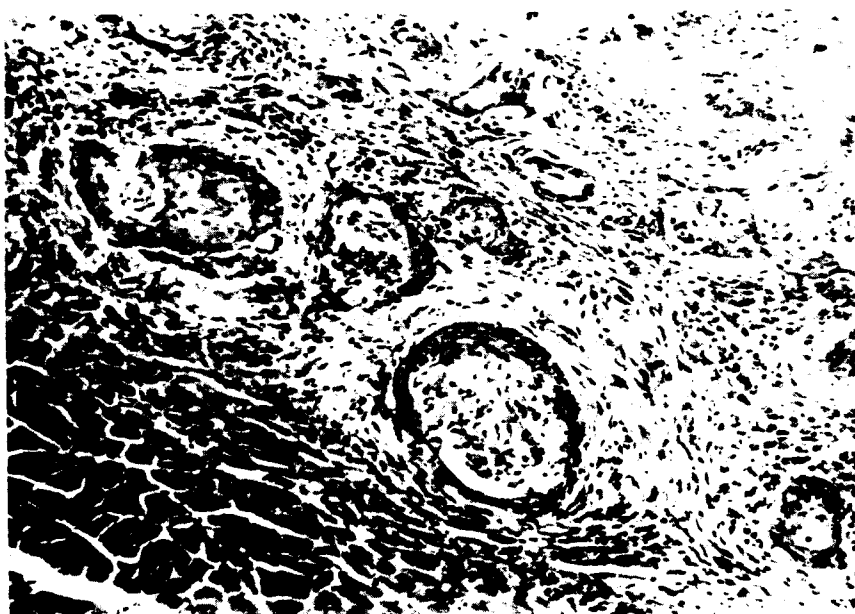


FIGURE 3. Squamous cell carcinoma of the oral cavity in a female Wistar rat exposed to benzene by ingestion. (Hematoxylin and eosin stain. Original magnification $\times 200$; reduced by 15%.)

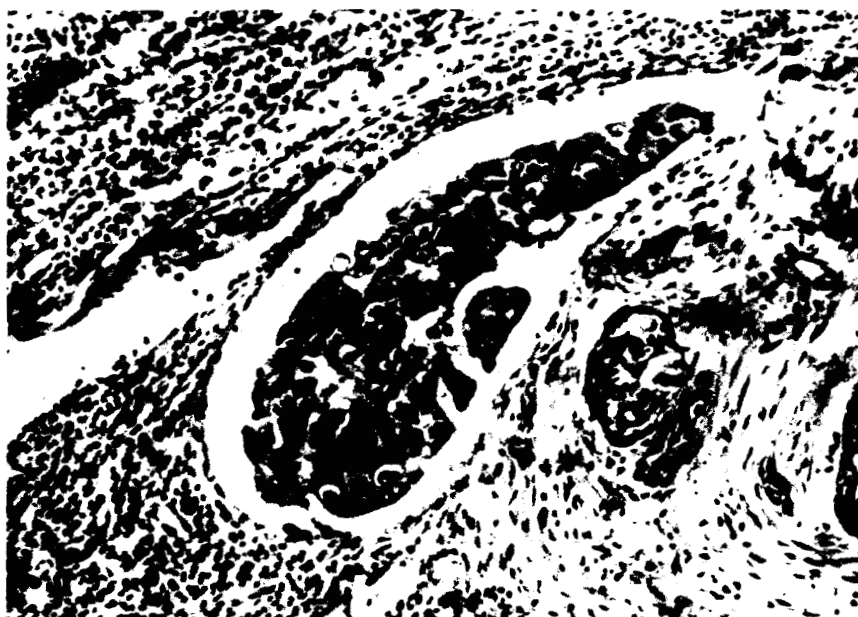


FIGURE 4. Lymph node metastasis of a squamous cell carcinoma of the oral cavity in a male Wistar rat exposed to benzene by ingestion. (Hematoxylin and eosin stain. Original magnification $\times 200$; reduced by 15%.)



FIGURE 5. Carcinoma of the nasal cavities in a male Wistar rat exposed to benzene by ingestion. (Hematoxylin and eosin stain. Original magnification $\times 200$; reduced by 15%.)

TABLE 12. Benzene: Total Tumors in Mice Treated by Ingestion (Stomach Tube) (Exp. BT 908)

Group No.	Dose (mg/kg)	Animals		Percentage of Animals with Tumors		No. of Malignant Tumors per 100 Animals
		Sex	No. at Start	TBMT ^a	MT ^b	
I	500	M	40	60.0	35.0	40.0
		F	40	80.0	70.0	80.0
		M + F	80	70.0	52.5	60.0
II	Olive oil (Control)	M	40	37.5	22.5	22.5
		F	40	40.0	27.5	21.5
		M + F	80	38.1	25.0	25.0

^a Total benign and malignant tumors.^b Malignant tumors.

are mammary carcinomas (in females), lung tumors, leukemias and hepatomas. Moreover, a variety of other miscellaneous tumors were also observed.^{6,7}

The data on the incidence of the total and most frequent tumors are given in **TABLES 12** and **13**. Benzene increased the incidence of total benign and malignant tumors, and of malignant tumors, both in males and females. Exposure to benzene increased the incidence of mammary carcinomas (**Figs. 6** and **7**) in female mice

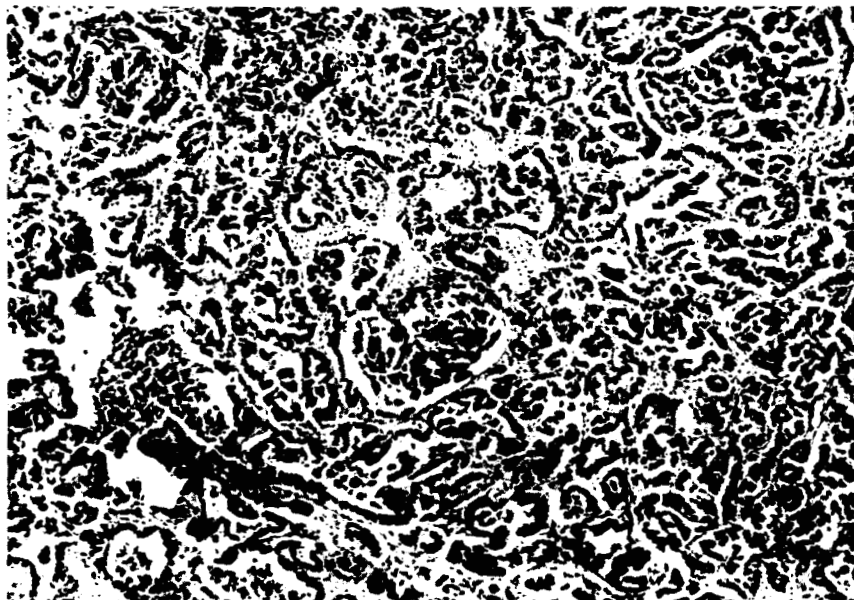


FIGURE 6. Mammary adenocarcinoma (type B) in a female Swiss mouse exposed to benzene by ingestion. (Hematoxylin and eosin stain. Original magnification $\times 80$; reduced by 15%.)

TABLE 13. Benzene: Mammary Carcinomas, Pulmonary Tumors, Leukemias, and Hepatomas in Mice Treated by Ingestion Stomach Tube (Exp. BT 908)

Group No.	Dose (mg/kg)	Animals		Percentage of Animals with Tumors			
		Sex	No. at Start	Mammary Carcinomas	Pulmonary Tumors	Leukemias ^a	Hepatomas
I	500	M	40	—	42.5	12.5	7.5
		F	40	47.5	37.5	20.0	—
II	Olive oil (Control)	M + F	80	23.7	40.0	16.2	3.7
		M	40	2.5	7.5	12.5	5.0
		F	40	5.0	10.0	20.0	—
		M + F	80	3.7	8.7	16.2	2.5

^a The term "leukemia" includes a variety of hemolymphoreticular neoplastic diseases at different sites.

TABLE 14. Benzene: Distribution of Different Types of Pulmonary Lesions in Mice Treated by Ingestion (Stomach Tube) (Exp. BT 908)

Group No.	Dose (mg/kg)	Sex	No. at Start	Percentage of Animals with Pulmonary Tumors				
				Total	Adenomatous Hyperplasia—Early Adenomas	Adenomas	Atypical Adenomas	Adenocarcinomas
I	500	M	40	42.5	2.5	22.5	15.0	2.5
		F	40	37.5	—	22.5	15.0	—
II	Olive oil (Control)	M + F	80	40.0	1.2	22.5	15.0	1.2
		M	40	7.5	—	5.0	2.5	—
		F	40	10.0	—	10.0	—	—
		M + F	80	8.7	—	7.5	1.2	—

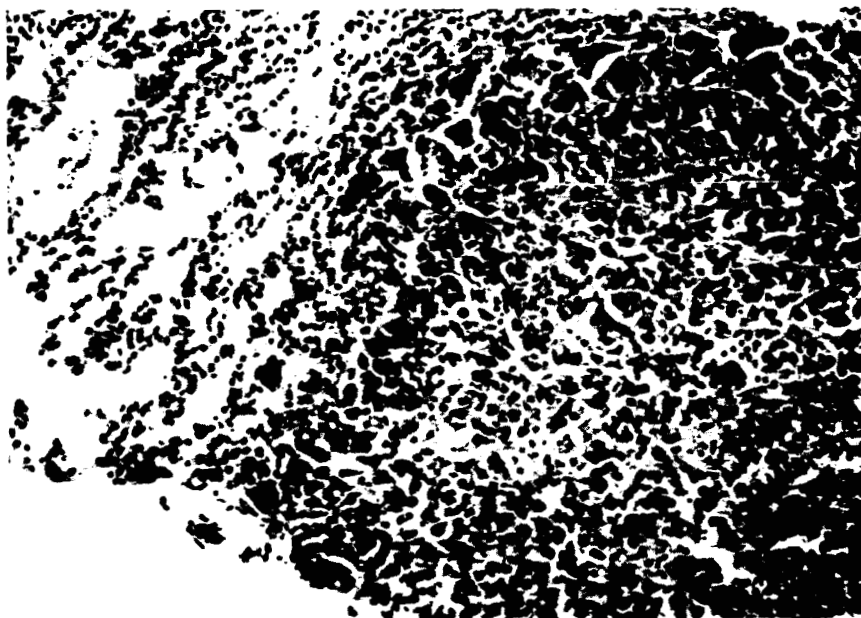


FIGURE 7. Lung metastasis of a mammary adenocarcinoma (type B) in a female Swiss mouse exposed to benzene by ingestion. (Hematoxylin and eosin stain. Original magnification $\times 200$; reduced by 15%.)

and of lung tumors (FIGS. 8 and 9) in males and females (TABLE 13). The distribution of different types of pulmonary tumors is shown in TABLE 14. Benzene also induced dysplasias and carcinomas (FIG. 10) of the Zymbal glands (TABLE 15). No increase was observed in the incidence of leukemias after benzene treatment (TABLE 13).

TABLE 15. Benzene: Zymbal Gland Carcinomas and Correlated Precancerous Lesions in Mice Treated by Ingestion (Stomach Tube) (Exp. BT 908)

Group No.	Dose (mg/kg)	Animals		Percentage of Animals with Lesions		
		Sex	No. at Start	Total	Dysplasias	Carcinomas
I	500	M	40	17.5	7.5	10.0
		F	40	12.5	10.0	2.5
		M + F	80	15.0	8.7	6.2
II	Olive oil (Control)	M	40	—	—	—
		F	40	—	—	—
		M + F	80	—	—	—

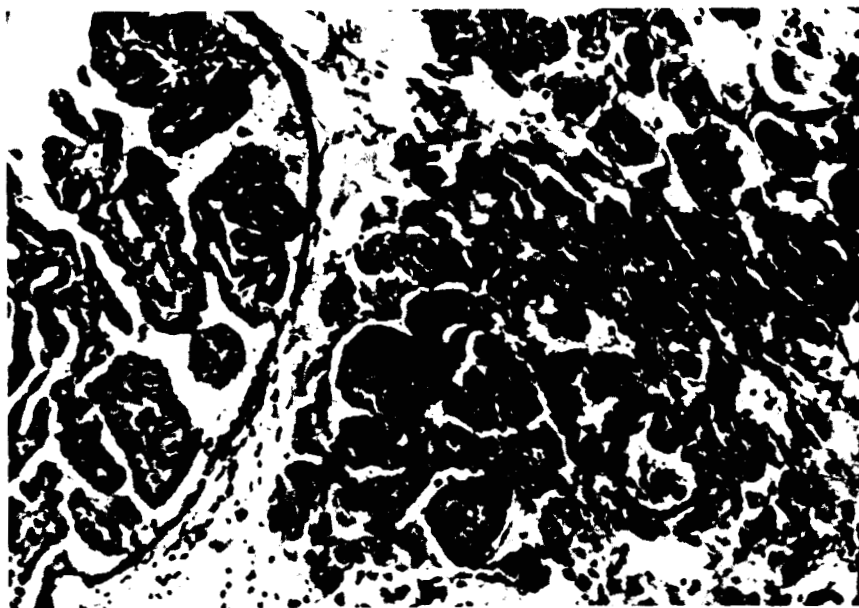


FIGURE 8. Pulmonary atypic adenoma in a female Swiss mouse exposed to benzene by ingestion. (Hematoxylin and eosin stain. Original magnification $\times 200$; reduced by 15%.)

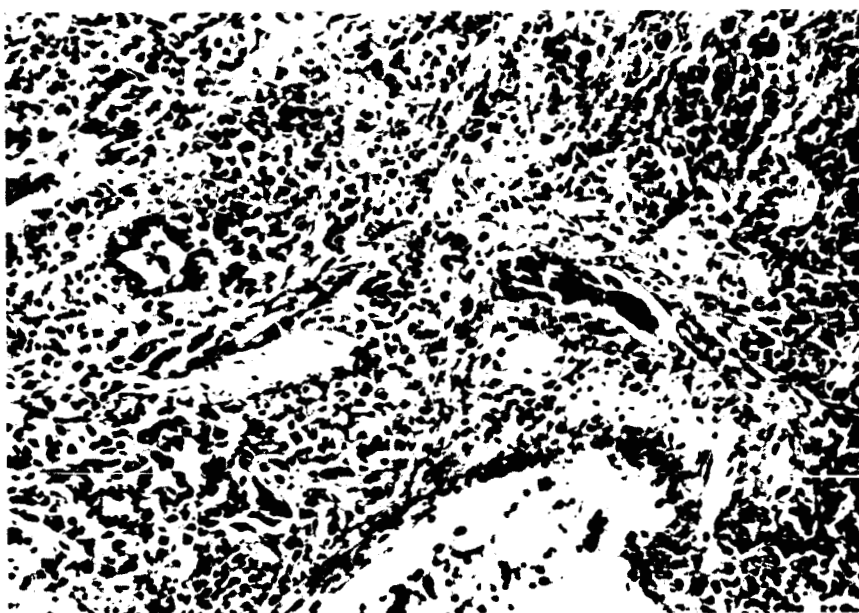


FIGURE 9. Pulmonary adenocarcinoma in a male Swiss mouse exposed to benzene by ingestion. (Hematoxylin and eosin stain. Original magnification $\times 200$; reduced by 15%.)



FIGURE 10. Zymbal gland squamous cell carcinoma in a female Swiss mouse exposed to benzene by ingestion. (Hematoxylin and eosin stain. Original magnification $\times 200$; reduced by 15%.)

CONCLUSIONS

As was shown in experiments performed on Sprague-Dawley rats, benzene is a multipotential carcinogen in Wistar rats and Swiss mice.

Of particular interest is the sharp increase in the incidence of mammary carcinomas in female mice and of lung tumors in male and female mice treated with the compound.

The experimental evidence of benzene carcinogenicity, based on the BT experimental project, is summarized in **TABLE 16**.

SUMMARY

Wistar rats and Swiss mice were treated by ingestion (stomach tube) with benzene in olive oil at a dose of 500 and 0 mg/kg b.w. once daily, 4–5 days weekly, for 104 weeks (rats) or for 78 weeks (mice).

In Wistar rats, benzene caused Zymbal gland carcinomas, carcinomas of the oral cavity, and carcinomas of the nasal cavities, and an increase in the incidence of total malignant tumors.

In Swiss mice, benzene produced Zymbal gland carcinomas and dysplasias and an increase in the incidence of mammary carcinomas (in females), lung tumors, and total malignant tumors.

TABLE 16. Evidence of Benzene's Carcinogenicity on the Basis of the BT Experimental Project

Tumors	Animals		
	Sprague-Dawley Rat ^a	Wistar Rat	Swiss Mouse
Total number of malignant tumors	+	+	+
Carcinomas of Zymbal gland	+	+	+
Carcinomas of oral cavity	+	+	
Carcinomas of nasal cavities	+	+	
Carcinomas of the skin	+		
Carcinomas of the forestomach	+		
Carcinomas of the mammary glands	+		+
Hepatomas	+		
Angiosarcomas of the liver	+		
Hemolymphoreticular neoplasias	(+)		
Tumors of the lung			+

^a From Maltoni *et al.*³

These experiments further confirm that benzene is a multipotential carcinogen as was shown before by long-term bioassays performed on Sprague-Dawley rats in the same Experimental Unit.

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