Benzene, an Experimental Multipotential Carcinogen: Results of the Long-Term Bioassays Performed at the Bologna Institute of Oncology

by Cesare Maltoni, * Adriano Ciliberti, * Giuliano Cotti, * Barbara Conti, * and Fiorella Belpoggi *

In 1976, a systematic and integrated project of long-term carcinogenicity bioassays began at the Bentivoglio Experimental Unit of the Bologna Institute of Oncology. The Bologna experiments proved for the first time that benzene is an experimental carcinogen. These experiments demonstrated that benzene is carcinogenic when administered by ingestion and by inhalation and that it cause tumor in the various tested animal models (Sprague-Dawley rats, Wistar rats, Swiss mice, and RF/J mice). They also showed that benzene is a multipotential carcinogen, as it produces a variety of neoplasias in one or more of the tested animal models, including Zymbal gland carcinomas, carcinomas of the oral cavity, nasal cavities, skin, stomach, and mammary glands, as well as angiosarcomas of the liver, hemolymphoreticular neoplasias, tumors of the lung, and possibly hepatomas. The Bologna experiments also indicated a clear-cut dose-response relationship in benzene carcinogenesis.

This report presents the up-to-date results of the Bologna project. The need for more experimental research aimed at assessing the carcinogenic effects of low doses of benzene, of chemical mixtures containing benzene, and of benzene substitute is emphasized. Also recommended are more comprehensive epidemiological investigations, extended to all types of malignancies, with particular regard to lung carcinomas.

Introduction

Benzene has been produced industrially from coal since 1849 and from petroleum since 1941. At present the major source of benzene is petroleum. Benzene is one of the largely diffused and produced industrial compounds. It is a constituent of crude oil, it is present in gasoline and other fossil fuels, and it is currently produced at the rate of about 15 million tons per year (the major producers are the U.S., Japan, and Western Europe). The total global annual cycle of benzene is estimated to be 32 million tons per year (1).

The major use of benzene in past was in blends with gasoline. Although this use has been reduced in the United States, benzene is still extensively employed in many countries for the production of commercial gasoline. The benzene content in gasoline varies from country to country, and its range is estimated to be from 1 to 15%. Currently, benzene is used as a chemical intermediate for the production of many important industrial compounds, such as ethylbenzene (used in the production of styrene), phenol, cyclohexane, maleic anhydride, aniline, dichlorobenzenes, etc., which, in turn, supply numerous sectors of the chemical industry, especially those producing plastics, resins, elastomers, dyes, and pesticides. In the past, benzene was also used as a solvent for paints and rubber, in the production of rubber cement (widely used in the shoe and garment industries), and in the manufacture of artificial leather. It has also been used in medicine in the treatment of hemoblastomas (leukemias, polycytemia, and malignant lymphomas) and in veterinary medicine of disinfecting wounds.

Of the 32 million tons of benzene circulated globally per year, 4 million tons are estimated to be lost to the environment (1). The major source is motor vehicle emission and evaporation losses during handling, distribution, and storage of petrol (2). Burning wood and organic material also results in an appreciable release of benzene. Tobacco smoke contains benzene at levels of 47 to 64 ppm (3). It is believed that plant and animal matter also release benzene into the environment (4).

Population groups that may be exposed to benzene in-
clude workers engaged in its production; workers in chemical industries using benzene as an intermediate; workers in industries producing materials containing benzene as a constituent (gasoline), as a solvent (rubber cement), or as an impurity (i.e., industry toluene); people living near factories producing or using benzene, or compounds containing it; tobacco smokers; and the general population (particularly in industrialized towns), as benzene is contained in gasoline, drinking water, and many other goods and is highly volatile.

Prolonged exposure to benzene causes toxic effects on bone marrow, both in animals and humans. The toxic effects on the hematopoietic system in humans have been known for about 90 years and are well documented in the literature. The classical clinical finding in benzene hematotoxicity is a decrease in the various formed elements of circulating blood (pancytopenia) as a consequence of the decrease in identifiable granulocyte, erythrocyte, and platelet precursors within the bone marrow. The association between long-term exposure to benzene and the occurrence of leukemia was suggested as early as 1928 by Delore and Borgomano (5), who reported a lymphoblastic leukemia in a worker who had been exposed to benzene for 5 years.

In spite of its industrial importance, widespread use, ubiquitous diffusion, the large number of people potentially exposed, and the early reports of occupational leukemias, there were no adequate epidemiological investigations nor adequate experimental research on benzene until the mid-1970s.

Knowledge of Benzene Carcinogenicity until the Mid-1970s

Human data are based, almost exclusively, on reports of a series of clinical cases of leukemia (generally in individuals with a history of benzene myelotoxicity) and on more indirect epidemiological investigation on the correlation between benzene exposure and the incidence of leukemias.

Since the first report of Delore and Borgomano (5), many leukemias in people exposed to benzene were the subject of case reports. Vigliani in 1976 (6) stated that "... an approximate estimation of the available literature, including some unpublished North Italian cases of which we have knowledge, puts the number of known cases of leukemia attributed to benzene at, at least, 150." These cases were collected and reviewed by Goldstein (7) (Table 1).

Acute myelogenous leukemia has been the most frequent form of leukemia associated with benzene exposure. Other forms of leukemia that have also been associated with benzene are erythroleukemia, acute monocytic leukemia, chronic myelogenous leukemia, myelofibrosis and myeloid metaplasia, thrombocytopenia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and lymphomas of various types.

The majority of the cases have been found in Italy (6, 8), France (9-14), and Turkey (15, 16). Most of the cases occurred among shoemakers and garment industry workers handling rubber cement.

The variety in type and distribution of the described leukemias in the major case reports (with a more or less pronounced prevalence of acute myelogenous leukemia) may be due to several factors: a) difference in level of exposure; b) exposure in association with other agents, with toxic effects on hematopoietic tissues; c) individual responsiveness; and d) differences in the morphological interpretation of the hemopathological pictures, which vary with time, country, and training.

All of the previously mentioned reports deal with a limited series of cases, and therefore the cases are fragmented into a large number of reports (Table 1). In most of these reports, the causal relation between benzene exposure and leukemia is developed more from the history of the single cases, showing characteristic sequence, benzene exposure-myelotoxicity-leukemia, than from statistical and biological evaluation.

Moreover, the indirect results of one epidemiological investigation performed in Japan, were reported. Ishimaru and co-workers (17) examined 308 cases of leukemia that occurred among adult survivors of the atomic bomb explosions at Hiroshima and Nagasaki and compared them with 308 matched controls exposed to the same amount of ionizing radiation from the bomb. They found that the risk of leukemia was approximately 2.5 times higher among those with the history of a probable exposure to benzene or its derivatives and to medical X-rays.

Benzene was shown to be genotoxic to human blood cells. Increased rates of both stable and unstable chromosome changes have been described both in lymphocytes and bone marrow cells of patients with benzene hemopathy and in lymphocytes of workers with past exposure to benzene but without signs of poisoning (18-24). Chromosome damage from benzene can persist for years in long-lived lymphocytes and may result in the formation of abnormal cell clones in the absence of any sign of disease (21, 22). Increased chromosome aberrations of blood lymphocytes have also been reported in workers exposed to

| Type                                      | No. of individuals | No. of reports |
|-------------------------------------------|--------------------|----------------|
| Acute myelogenous leukemia                 | 58                 | 28             |
| Erythroleukemia                           | 16                 | 10             |
| Acute monocytic leukemia                   | 3                  | 2              |
| Chronic myelogenous leukemia               | 27                 | 7              |
| Myelofibrosis and myeloid metaplasia       | 7                  | 5              |
| Thrombocytopenia                          | 1                  | 1              |
| Acute lymphoblastic leukemia               | 8                  | 4              |
| Chronic lymphocytic leukemia               | 9                  | 7              |
| Lymphomas and correlated disorders         | 14                 | 7              |
| Total                                     |                    | 143            |

Table 1. Case reports of hemolymphoreticular neoplasia and correlated diseases observed in individuals exposed to benzene reported in scientific literature until the mid-1970s (7).
less than 25 ppm of benzene (25–27). There have been a number of reports of cases of benzene pancytopenia in which the observed bone marrow cellular atypias were progressing toward acute leukemias.

The available experimental data prior to 1976, summarized in Table 2, are scanty and insubstantial (28–32). In IARC Monograph No. 7 (32), it was concluded that “benzene has been tested only in mice by subcutaneous injection and skin application. The data reported do not permit the conclusion that carcinogenic activity has been demonstrated.”

Knowledge of Benzene Carcinogenicity from the Mid-1970s to the Present

A systematic integrated experimental project (the largest up to present) of long-term carcinogenicity bioassays on benzene began in April 1976 at the Bentivoglio (BT) Experimental Unit of the Bologna Institute of Oncology. The project was aimed at studying the carcinogenic effects of benzene administered by different routes (ingestion and inhalation) at different daily doses/concentrations on animals of different species and strains (Sprague-Dawley and Wistar rats and Swiss and RF/J mice) and of different ages at the start of the treatment. As early as November–December 1977, preliminary results were published (33) showing that benzene was producing in Sprague-Dawley rats Zymbal gland carcinomas, an increase of other solid tumors and a marginal increase of malignant hemolymphoreticular neoplasias. Since then, the data of this project, have shown that benzene has carcinogenic effects when given by inhalation and by ingestion, it causes tumors in all tested animal species and strains, it is a multipotential carcinogen, as it produces a large variety of neoplasias, and there is a clear-cut dose-response relationship in benzene carcinogenesis. The data have been summarized, from 1977 to 1987, in various publications (34–45).

Further experimental bioassays were then performed in other laboratories. Bone marrow hyperplasia, thymic lymphoma (6/40), plasmacytoma (1/40), and leukemia (1/40) were reported in C57BL/6J mice exposed to air contain-

| Animals | Treatment and other experimental details | Results | Observations | Reference |
| --- | --- | --- | --- | --- |
| Mice Albino M,F 33 T | SC injection of 0.001 mL of benzene in 0.1 mL of olive oil, once weekly, for 17–21 weeks (total dose, about 1 mg/kg body weight) | 8 Leukemias (from 4-8 months from the starting of the treatment) | No control group | (28) |
| Mice F ? 30 T | SC injection of 0.001 mL of benzene in 0.1 mL of sesame oil | 6 Leukemias (30%) (from 200-300 days of age) | Leukemia increase in treated animals is not statistically significant | (29) |
| Mice DBA2 M 30 T C3H 30 T C57BL6 30 T | SC injection of 0.001 mL of benzene in 0.1 mL of olive oil, for all life span | 0 | Maximum survival of DBA2, C3H and C57BL/6 mice: 730 days | (30) |
| AKR M 30 T | SC injection of 0.001 mL of benzene in 0.1 mL of olive oil, for 10 weeks | 16 Leukemias (between the 7 and 16 months of treatment), 8 animals dead before the month 9 of treatment | Major survival of animals of control group | |
| Mice Swiss M,F 10 T | SC injection of 0.001 mL of benzene in 0.1 mL of olive oil, for 10 weeks | 5 SC sarcomas (at autopsy, performed between days 162 and 253 from start of treatment) | 2 Animals dead within the first 8 weeks of treatment | (31) |
| Mice Various M,F Very many Rabbits | Skin applications | No effects | Nonsystematic and non-ad hoc planned experiments | Many |

*T, treated; C, control.
ing 300 ppm benzene for 6 hr/day, 5 days/week, for 488 days as compared with an incidence of 2/40 lymphomas (nonthymic) in the controls (46). Myelogenous leukemia occurred in 2/40 CD-1 mice exposed to air containing 300 ppm benzene for 6 hr/day, 5 days/week, for life (47). The results of these two studies, because of the small number of animals used and the marginal increase of the observed hemolymphoreticular neoplasias, do not allow establishment of a positive association between the onset of the neoplasias and the benzene exposure.

Cronkite (48,49) showed that female mice exposed at 300 ppm (6 hr/day, 5 days/week for 16 weeks, after which exposures was stopped) exhibited increased incidence of leukemias (8/88 versus 20/89) largely due to thymic lymphomas (1/88 versus 10/89). In addition, Zymbal gland neoplasias (1/88 versus 16/89) and ovarian tumors (0/88 versus 8/89) were increased.

NTP performed a long-term carcinogenicity bioassay on F344/N rats and B6C3F1 mice (50). Fifty male and fifty female/dose level were gavaged 5 days/week for 103 weeks. Doses of 0, 50, 100, or 200 mg/kg body weight benzene in corn oil (0.5 mL/kg) were administered to male rats. Doses of 0, 25, 50, or 100 mg/kg benzene in corn oil were administered to female rats and to male and female mice. The results of this experiment confirm the findings of Bologna-BT project, i.e., that benzene is a multipotential carcinogen. A definite or marginally increased incidence of the following tumors was found in benzene-exposed animals of one or both sexes: tumors of Zymbal glands, oral cavity, and skin in rats; tumors of Zymbal glands, hemolymphoreticular tissues, lungs, Hardieian glands, mammary glands, preputial glands, forestomach, ovary, and liver in mice.

Recent epidemiological studies of small cohorts exposed to benzene have demonstrated a causal association with leukemia (51–58). Infante et al. (51) reported an increased risk of leukemias among workers at three rubber hydrochloride plants (in two Ohio locations) who were exposed to benzene in the years 1940 to 1949. In that investigation, the vital status of 75% of the population was ascertained. The level of exposure was estimated to be not greater than the standards at that time would have allowed. Rinsky et al. (59) reported the findings of a more extensive investigation on the same rubber workers. The major findings of this important study may be summarized as follows: a) the workers were exposed to only one agent that has been associated with blood dyscrasias, i.e., benzene; b) exposure data, uncommonly complete throughout the study period (1940–1975), indicated that the exposures of the workers were, for the most part, within limits permissible at the time (these limits are not greatly higher than the current legal standards); and c) the vital status (alive, dead, and cause of death) for 98% of the study population was ascertained. There were seven deaths from leukemia (myelocytic or monocytic) among 748 workers; this rate is 5.6-fold greater than would be expected in a comparable population. For workers exposed 5 years or more, there was a 21-fold increase of death from leukemia. In 1986, Rinsky et al. (57,58) reexamined the updated mortality on the same cohorts and calculated a cumulative benzene exposure index (ppm × years) for each cohort member. These authors found that the standard mortality ratio (SMR) for leukemia was 328 and for multiple myeloma was 398. With stratification of the cohort by cumulative exposure, the SMRs for leukemia increased from 105 in workers with less than 40-ppm years exposure, to 314 in workers with 40- to 199-ppm years, to 1757 in those with from 200- to 399-ppm years, and to 4535 in those with 400-ppm years or more.

In 1985, Maltoni et al. reported the first experimental evidence of the carcinogenicity of benzene-correlated compounds, namely toluene and xylene (44). These results have been recently confirmed by the same authors by further experiments whose results are now in publication.

The history of benzene carcinogenicity is summarized in Table 3.

This report presents the up-to-date results of the experiments on benzene carcinogenicity performed by the Institute of Oncology of Bologna (BT experimental project).

Materials and Methods

The plan of the experiments is shown in Tables 4–10. The bioassay on RF/J Mice (Table 10) is part of a larger experiment aimed at studying the effect of 10% ethyl alcohol, administered instead of drinking water, on benzene carcinogenesis (Table 11). (The results of the whole experiment are now being submitted for publication.) Data on test compounds and test animals are presented in Table 12. Details on the conduct of the experiments are as follows:

- The animals were exposed by inhalation in air, 4 to 7 hr/day, 5 days/week, for 15 and 104 weeks. The chambers for inhalation exposure were stainless steel, with two glass doors, and measured $135 \times 98 \times 65$ cm. The volume was 860 L. Continuous air flow provided 12 to 15 air changes per hour. Before introduction, air was filtered and the chamber arrangement was such that air flowed from one part of the chamber to the other with-

### Table 3. History of benzene carcinogenicity.

| Year   | Report                                                                 |
|-------|------------------------------------------------------------------------|
| 1928  | First report on an acute leukemia following benzene intoxication (5)   |
| 1960s | Reports of cases of leukemias among workers (mainly shoemakers) heavily exposed to benzene, in Italy (8) |
| 1970s | Reports of cases of leukemias among workers (mainly shoemakers) heavily exposed to benzene, in Italy (15,16) |
| 1977  | First evidence of carcinogenicity of benzene in experimental animals (rats) (59) |
| 1977–1983 | Experimental evidence showing that benzene is a multipotential carcinogen in rodents (rats and mice) (44) |
| 1977–1986 | Epidemiological evidence of benzene leukemogeni- |
| 1983–1987 | First experimental evidence of carcinogenicity of benzene correlated compounds (toluene and xylene) (44) |
Table 4. Plan of experiments on benzene carcinogenicity: experiment BT 901.*

| Group no. | Dose               | Sprague-Dawley rats, 13 weeks old at start |          |          | Total |
|-----------|--------------------|--------------------------------------------|----------|----------|-------|
|           |                    | M   | F   | Total   |
| I         | 250 mg/kg body weight | 35  | 35  | 70      |
| II        | 50 mg/kg body weight  | 30  | 30  | 60      |
| III       | 0 (Controls)*       | 30  | 30  | 60      |
| Total     |                    | 95  | 95  | 190     |

*Exposure by ingestion (stomach tube) in olive oil, once daily, 4-5 days weekly, for 52 weeks. Duration of the biophase, life-span.

Table 5. Plan of experiments on benzene carcinogenicity: experiment BT 902.*

| Group no. | Dose               | Sprague-Dawley rats, 7 weeks old at start |          |          | Total |
|-----------|--------------------|-------------------------------------------|----------|----------|-------|
|           |                    | M   | F   | Total   |
| I         | 500 mg/kg body weight | 40  | 40  | 80      |
| II        | 0 (Controls)*       | 50  | 50  | 100     |
| Total     |                    | 90  | 90  | 180     |

*Exposure by ingestion (stomach tube) in olive oil, once daily, 4-5 days weekly, for 104 weeks. Duration of the biophase, life-span.

Table 6. Plan of experiments on benzene carcinogenicity: experiments BT 901, BT 902.*

| Experiment and group no. | Dose               | Treatment | Sprague-Dawley rats, 7 and 13 weeks old at start |          |          | Total |
|--------------------------|--------------------|-----------|-------------------------------------------------|----------|----------|-------|
|                          |                    | Length, weeks | M   | F   | Total   |
| BT 902 I                 | 500 mg/kg body weight | 104       | 40  | 40  | 80      |
| BT 901 I                 | 250 mg/kg body weight | 52        | 35  | 35  | 70      |
| BT 901 II                | 50 mg/kg body weight | 52        | 30  | 30  | 60      |
| BT 901 III               | 0 (Controls)*      | 30        | 30  | 60      |
| BT 902 II                | 0 (Controls)*      | 50        | 50  | 100     |
| Total                    |                    | 185       | 185 | 370     |

*Exposure by ingestion (stomach tube) in olive oil, once daily, 4-5 days weekly. Duration of the biophase, life-span.

out recirculation. The internal pressure was about 1 mm Hg less than that of the room where the chamber was situated, to avoid any possible contamination of the outside environment. Chambers were maintained at 21°C ± 3°C and at 50% ± 10% relative humidity. Lighting was provided by room light. The chambers were cleaned at monthly intervals. Exposure chambers were provided with a fixed point matrix for checking the distribution of the test substance. During treatment the distribution was continuously monitored by gas chromatographs.

- The animals were exposed by ingestion (stomach tube, made of stainless steel), once daily, 4 to 5 days/week, for 52 (Sprague-Dawley rats, RF/J mice), 78 (Swiss mice) and 104 weeks (Sprague-Dawley and Wistar rats).
- 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; see below.
- The housing and the diet of the animals were the same adopted in the BT Experimental Unit during the last 15 years.
- All the experiments were performed with the same highly standardized procedures in order to allow comparison.

The tissues and organs submitted to histopathological examination were the following: subcutaneous lymph nodes, brain and cerebellum, pituitary, Zymbal glands, inter- scalpular 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, esophagus, mesenteric lymph nodes, stomach, various segments of the intestine, bladder, uterus, gonads, any other organs with pathological lesions, and, only for experiment BT 909, lachrymal and preputial glands.
Table 7. Plan of experiments on benzene carcinogenicity: experiments BT 4004, 4006.

| Group no. | Concentration | Treatment | Schedule | Sprague-Dawley rats, 13 weeks old, breeder (B) and 12-day embryo (E) |
|-----------|---------------|-----------|----------|---------------------------------------------------------------------|
|           |               |           | Age      | M | F | Total |
| I         | 200 ppm       | 4 hr/day, 5 days/week, 7 weeks | B | 54<sup>a</sup> | (22)<sup>f</sup> | 54 |
| I         | 300 ppm       | 7 hr/day, 5 days/week, 85 weeks<sup>b</sup> | E | 75 | 65 | 140 |
| II        | 200 ppm       | 4 hr/day, 5 days/week, 7 weeks | E | 70 | 59 | 129 |
| II        | 300 ppm       | 7 hr/day, 5 days/week, 85 weeks<sup>b</sup> | E | 70 | 59 | 129 |
| III       | 200 ppm       | 4 hr/day, 5 days/week, 7 weeks | E | 70 | 59 | 129 |
| IV        | 0             |           |           | B | 60<sup>d</sup> | 60 |
| V         | 0             |           |           | E | 158 | 149 | 307 |
| Total     |               |           |           |   | 303 | 387 | 690 |

<sup>a</sup>Exposure by inhalation for 15 and 104 weeks. The embryos were exposed transplacently during pregnancy, and the offspring were exposed concurrently during weaning by inhalation and possibly by ingestion via milk.

<sup>b</sup>Total period of exposure, 104 weeks.

<sup>c</sup>Total period of exposure, 15 weeks.

<sup>d</sup>Total breeders.

<sup*e</sup>Pregnant breeders.

Table 8. Plan of experiments on benzene carcinogenicity: experiment BT 907.

| Group no. | Dose               | Wistar rats, 7 weeks old at start |
|-----------|--------------------|-----------------------------------|
|           |                    | M | F | Total |
| I         | 500 mg/kg body weight | 40 | 40 | 80 |
| II        | 0 (Controls)<sup>b</sup> | 40 | 40 | 80 |
| Total     |                    | 80 | 80 | 160 |

<sup>a</sup>Exposure by ingestion (stomach tube) in olive oil, once daily, 4-5 days weekly, for 104 weeks. Duration of the biophase, life-span.

<sup>b</sup>Olive oil alone.

Table 9. Plan of experiments on benzene carcinogenicity: experiment BT 908.

| Group no. | Dose               | Swiss mice, 7 weeks old at start |
|-----------|--------------------|----------------------------------|
|           |                    | M | F | Total |
| I         | 500 mg/kg body weight | 40 | 40 | 80 |
| II        | 0 (Controls)<sup>b</sup> | 40 | 40 | 80 |
| Total     |                    | 80 | 80 | 160 |

<sup>a</sup>Exposure by ingestion (stomach tube), in olive oil, once daily, 4-5 days weekly, for 78 weeks. Duration of the biophase, life-span.

<sup>b</sup>Olive oil alone.
Table 12. Test compound and test animals.

| Test compound | Purity  | Vehicle                      | Test animals                                                                 |
|---------------|---------|------------------------------|-------------------------------------------------------------------------------|
| Benzene       | 99.93%  | (Ingestion experiments) extra-virgin olive oil, with no detectable levels of pesticides, supplied by Olearia Toscana. | Male and female Sprague-Dawley rats 7, 13 weeks old, and 12-day embryos at start of the experiment |
| Paraffin      | 0.06%   | -                            | Male and female Wistar rats, 7 weeks old at start of the experiment           |
| Toluene       | 0.01%   | -                            | Male and female Swiss mice, 7 weeks old at start of the experiment            |

Results

Experiment on Sprague-Dawley Rats (Tables 13–25)

The most frequent tumors in this strain of rats, on the basis of the literature and of the historical controls of the BT Experimental Unit, are mammary tumors, malignant hemolymphoreticular neoplasias (leukemias), pheochromocytomas, and pheochromoblastomas. Moreover a variety of other miscellaneous tumors were also observed (59).

The administration of benzene by ingestion is associated with an increase of total malignant tumors and carcinomas of the Zymbalg glands (with sebaceous and squamous patterns), oral cavity, nasal cavities, skin (of different histotypes), forestomach (together with an increase of acanthomas and dysplasias), and with liver angiosarcomas, and a marginal increase of carcinomas of the mammary glands, hepatomas, and leukemias.

The administration of benzene by inhalation is associated with an increase of total malignant tumors and carcinomas of the Zymbalg glands and oral cavity, and with a marginal increase of carcinomas of the nasal cavities, mammary glands, and hepatomas.

Table 13. Experiment BT 901: incidence of total tumors in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 52 weeks.

| Group no. | Dose, mg/kg | Animals | % of animals bearing tumors | No. of malignant tumors per 100 animals |
|-----------|-------------|---------|----------------------------|----------------------------------------|
|           |             | Sex     | No. at start | TBMT<sup>a</sup> | MT<sup>b</sup> |                          |
| I         | 250         | M       | 35           | 37.1            | 20.0            | 22.8                     |
|           |             | F       | 35           | 65.7            | 42.8            | 60.0                     |
|           |             | M+F     | 70           | 51.4            | 31.4            | 41.4                     |
| II        | 50          | M       | 30           | 33.3            | 3.3             | 3.3                      |
|           |             | F       | 30           | 76.7            | 30.0            | 33.3                     |
|           |             | M+F     | 60           | 55.0            | 16.7            | 18.3                     |
| III       | Olive oil   | M       | 30           | 23.3            | 3.3             | 3.3                      |
|           | (Controls)  | F       | 30           | 60.0            | 23.3            | 23.3                     |
|           |             | M+F     | 60           | 41.7            | 13.3            | 13.3                     |

<sup>a</sup>Total benign and malignant tumors.  
<sup>b</sup>Malignant tumors.

Table 14. Experiment BT 901: incidence of mammary tumors, leukemias, pheochromocytomas, and pheochromoblastomas in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 52 weeks.

| Group no. | Dose, mg/kg | Animals | Mammary tumors | % of animals bearing tumors |
|-----------|-------------|---------|----------------|----------------------------|
|           |             | Sex     | No. at start | BMT<sup>a</sup> | MT<sup>b</sup> | Leukemias | Pheochromocytomas | Pheochromoblastomas |
| I         | 250         | M       | 35           | 5.7             | 20.0            | 11.4        | 2.9             | 2.9             |
|           |             | F       | 35           | 45.7            | 20.0            | 2.9         | 2.9             | 2.9             |
|           |             | M+F     | 70           | 25.7            | 10.0            | 7.1         | 2.9             | 2.9             |
| II        | 50          | M       | 30           | 20.0            | -               | -           | -               | -               |
|           |             | F       | 30           | 73.3            | 13.3            | 6.7         | 3.3             | 3.3             |
|           |             | M+F     | 60           | 46.7            | 6.7             | 3.3         | 1.7             | 3.3             |
| III       | Olive oil   | M       | 30           | 3.3             | -               | -           | 3.3             | 3.3             |
|           | (Controls)  | F       | 30           | 53.3            | 13.3            | 3.3         | 3.3             | 3.3             |
|           |             | M+F     | 60           | 28.3            | 6.7             | 1.7         | 3.3             | 3.3             |

<sup>a</sup>Benign and malignant tumors.  
<sup>b</sup>Malignant tumors (carcinomas, carcinosarcomas).
Table 15. Experiment BT 901: incidence of Zymbal gland carcinomas, auricular duct carcinomas, nasal cavities carcinomas, and oral cavity carcinomas in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 52 weeks.

| Group no. | Dose, mg/kg | Sex | No. at start | Zymbal gland carcinomas | Auricular duct carcinomas | Nasal cavity carcinomas | Oral cavity carcinomas | Total |
|-----------|-------------|-----|--------------|-------------------------|--------------------------|------------------------|------------------------|-------|
| I         | 250         | M   | 35           |                         | 22.9                     |                        | 5.7                    | 28.6  |
|           |             | F   | 35           |                         | 11.4                     |                        | 2.9                    | 14.3  |
| II        | 50          | M   | 30           |                         | 6.7                      |                        |                        | 6.7   |
|           |             | F   | 30           |                         | 3.3                      |                        |                        | 3.3   |
| III       | Olive oil   | M   | 30           |                         |                          |                        |                        |       |
| (Controls)|             | F   | 30           |                         |                          |                        |                        |       |
|           |             | M+F | 60           |                         |                          |                        |                        |       |

Table 16. Experiment BT 902: incidence of total tumors in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

| Group no. | Dose, mg/kg | Sex | No. at start | % of animals bearing tumors | No. of malignant tumors per 100 animals |
|-----------|-------------|-----|--------------|-----------------------------|---------------------------------------|
| I         | 500         | M   | 40           | 92.5                        | 90.0                                  | 170.0                                |
|           |             | F   | 40           | 92.5                        | 87.5                                  | 147.0                                |
|           |             | M+F | 80           | 92.5                        | 88.7                                  | 158.7                                |
| II        | Olive oil   | M   | 50           | 58.0                        | 24.0                                  | 24.0                                 |
| (Controls)|             | F   | 50           | 60.0                        | 20.0                                  | 22.0                                 |
|           |             | M+F | 100          | 59.0                        | 22.0                                  | 23.0                                 |

Table 17. Experiment BT 902: incidence of mammary tumors, leukemias, pheochromocytomas, and pheochromoblastomas in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

| Group no. | Dose, mg/kg | Sex | No. at start | % of animals bearing tumors | Mammary tumors | Leukemias | Pheochromocytomas | Pheochromoblastomas |
|-----------|-------------|-----|--------------|-----------------------------|----------------|-----------|-------------------|---------------------|
| I         | 500         | M   | 40           | 7.5                         | BMT<sup>a</sup> | 2.5       | 10.0              | 2.5                 |
|           |             | F   | 40           | 32.5                        | MT<sup>b</sup> | 7.5       | 5.0               |                     |
|           |             | M+F | 80           | 20.0                        |               | 5.0       | 7.5               | 1.3                 |
| II        | Olive oil   | M   | 50           | 4.0                         |               | 6.0       | 40.0              |                     |
| (Controls)|             | F   | 50           | 42.0                        |               | 2.0       | 22.0              |                     |
|           |             | M+F | 100          | 23.0                        |               | 4.0       | 31.0              |                     |

<sup>a</sup>Total benign and malignant tumors.  
<sup>b</sup>Malignant tumors.  

Table 18. Experiment BT 902: incidence of Zymbal gland carcinomas, auricular duct carcinomas, nasal cavities carcinomas, and oral cavity carcinomas in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

| Group no. | Dose, mg/kg | Sex | No. at start | % of animals bearing tumors | Zymbal gland carcinomas | Auricular duct carcinomas | Nasal cavity carcinomas | Oral cavity carcinomas | Total |
|-----------|-------------|-----|--------------|-----------------------------|-------------------------|--------------------------|------------------------|------------------------|-------|
| I         | 500         | M   | 40           | 45.0                        | 7.5                     | 52.5                     | 2.5                    | 30.0                   | 105.0 |
|           |             | F   | 40           | 40.0                        | 2.5                     | 50.0                     | 92.5                   |                       |       |
|           |             | M+F | 80           | 42.5                        | 5.0                     | 51.2                     | 98.8                   |                       |       |
| II        | Olive oil   | M   | 50           | 2.0                         |                          |                          |                        |                        | 2.0   |
| (Controls)|             | F   | 50           |                            |                          |                          |                        |                        |       |
|           |             | M+F | 100          | 1.0                         |                          |                          |                        |                        | 1.0   |

<sup>a</sup>Benign and malignant tumors.  
<sup>b</sup>Malignant tumors (carcinomas, sarcomas).
Table 19. Experiment BT 902: incidence of skin carcinomas in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

| Group no. | Dose, mg/kg | Animals | % of animals bearing skin carcinomas |
|-----------|-------------|---------|-------------------------------------|
| I         | 500         | M       | 40                                  | 22.5 |
|           |             | F       | 40                                  |      |
|           |             | M+F     | 80                                  | 11.3 |
| II        | Olive oil   | M       | 50                                  |      |
|           | (Controls)  | F       | 50                                  | 2.0  |
|           |             | M+F     | 100                                 | 1.0  |

Table 20. Experiment BT 902: incidence of hepatic tumors in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

| Group no. | Dose, mg/kg | Animals | % of animals bearing hepatic tumors |
|-----------|-------------|---------|-------------------------------------|
|           |             | Hepatomas | Angiosarcomas                      |
| I         | 500         | M       | 40                                  | 7.5  |
|           |             | F       | 40                                  | 2.5  |
|           |             | M+F     | 80                                  | 5.0  |
| II        | Olive oil   | M       | 50                                  | 6.0  |
|           | (Controls)  | F       | 50                                  |      |
|           |             | M+F     | 100                                 | 3.0  |

Table 21. Experiment BT 902: incidence of forestomach lesions in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

| Group no. | Dose, mg/kg | Animals | % of animals bearing forestomach lesions |
|-----------|-------------|---------|----------------------------------------|
|           |             | Acanthomas and In situ Dysplasias | Carcinomas | Invasive Carcinomas |
| I         | 500         | M       | 40                                  | 25.0 |
|           |             | F       | 40                                  | 17.5 |
|           |             | M+F     | 80                                  | 21.3 |
| II        | Olive oil   | M       | 50                                  |      |
|           | (Controls)  | F       | 50                                  |      |
|           |             | M+F     | 100                                 |      |

Table 22. Experiments BT 4004, 4006: incidence of total tumors in Sprague-Dawley rats exposed to benzene by inhalation for 15 and 104 weeks.

| Group no. | Treatment | Schedule | Animals | % of animals bearing tumors |
|-----------|-----------|----------|---------|-----------------------------|
|           |           | Agea | Sex | No. at start | TBMTb | MTc | No. of malignant tumors per 100 animals |
| I         | 200       | B    | F   | 54          | 70.4  | 27.8 | 29.6 |
| II        | 200       | E    | M   | 75          | 56.0  | 30.7 | 37.3 |
|           | 200       | F    | 65          | 78.5  | 58.5 | 78.5 |
|           | 200       | M+F  | 140        | 66.4  | 43.6 | 56.4 |
| III       | 200       | E    | M   | 70          | 52.8  | 28.6 | 31.4 |
|           | 200       | F    | 59          | 78.0  | 45.8 | 50.8 |
|           | 200       | M+F  | 129        | 64.3  | 36.4 | 40.3 |
| IV        | 0         | B    | F   | 60          | 58.3  | 15.0 | 16.7 |
| V         | 0         | E    | M   | 158         | 44.9  | 17.1 | 18.3 |
|           |           | F   | 149        | 78.5  | 17.4 | 17.4 |

* B, breeders; E, embryos.
* Total benign and malignant tumors.
* Malignant tumors.
Table 23. Experiments BT 4004,4006: incidence of mammary tumors, leukemias, pheochromocytomas, and pheochromoblastomas in Sprague-Dawley rats exposed to benzene by inhalation for 15 and 104 weeks.

| Group no. | Treatment | Animals | Mammary tumors % | Pheochromocytomas % | Pheochromoblastomas % |
|-----------|-----------|---------|------------------|---------------------|----------------------|
|           | Concentration, ppm | Schedule | Age* | Sex | No. at start | BMT* | MT* | Leukemias | |
| I         | 200       | 4 hr/day, 5 days/week, 7 weeks | B F | 54 | 55.5 | 11.1 | — | 7.4 | — |
|           | 300       | 7 hr/day, 5 days/week, 85 weeks | E M | 75 | 8.0 | — | 8.0 | 8.0 | 1.3 |
| II        | 200       | 4 hr/day, 5 days/week, 7 weeks | F | 65 | 53.8 | 13.8 | — | 6.1 | 1.5 |
|           | 300       | 7 hr/day, 5 days/week, 85 weeks | M + F | 140 | 29.3 | 6.4 | 4.3 | 7.1 | 1.4 |
| III       | 200       | 4 hr/day, 5 days/week, 7 weeks | F | 59 | 62.1 | 13.6 | 6.8 | 8.5 | — |
|           | 300       | 7 hr/day, 5 days/week, 8 weeks | M + F | 129 | 34.1 | 6.2 | 6.2 | 14.7 | 1.6 |
| IV        | 0         | (Controls) | B F | 60 | 40.0 | 3.3 | 3.3 | 18.3 | — |
| V         | 0         | (Controls) | E M | 158 | 7.0 | 1.9 | 7.6 | 20.2 | 0.6 |
|           |           |         | F | 149 | 56.4 | 5.4 | 0.7 | 18.8 | 0.7 |
|           |           |         | M + F | 307 | 30.9 | 3.6 | 4.2 | 20.8 | 0.6 |

*B, breeders, E, embryos.

Table 24. Experiments BT 4004,4006: incidence of Zymbal gland carcinomas, auricolar duct carcinomas, nasal cavities carcinomas, and oral cavity carcinomas in Sprague-Dawley rats exposed to benzene by inhalation for 15 and 104 weeks.

| Group no. | Treatment | Animals | % of animals bearing tumors |
|-----------|-----------|---------|-----------------------------|
|           | Concentration, ppm | Schedule | Age* | Sex | No. at start | Zymbal gland carcinomas | Auricolar duct carcinomas | Nasal cavity carcinomas | Oral cavity carcinomas | Total |
| I         | 200       | 4 hr/day, 5 days/week, 7 weeks | B F | 54 | 5.5 | — | 1.8 | 3.7 | 11.1 |
|           | 300       | 7 hr/day, 5 days/week, 85 weeks | E M | 75 | 8.0 | — | 1.3 | 1.3 | 10.7 |
| II        | 200       | 4 hr/day, 5 days/week, 7 weeks | F | 65 | 12.3 | 1.3 | 3.1 | 15.4 | 30.8 |
|           | 300       | 7 hr/day, 5 days/week, 85 weeks | M + F | 140 | 10.0 | — | 2.1 | 7.9 | 20.0 |
| III       | 200       | 4 hr/day, 5 days/week, 7 weeks | F | 59 | 1.7 | — | 1.7 | 10.2 | 13.6 |
|           | 300       | 7 hr/day, 5 days/week, 8 weeks | M + F | 129 | 3.9 | — | 1.6 | 6.2 | 11.6 |
| IV        | 0         | (Controls) | B F | 60 | 1.7 | — | — | — | 1.7 |
| V         | 0         | (Controls) | E M | 158 | 1.3 | — | — | — | 1.3 |
|           |           |         | F | 149 | — | — | — | — | — |
|           |           |         | M + F | 307 | 0.7 | — | — | — | 0.7 |

*B, breeders, E, embryos.
Table 25. Experiments BT 4004, 4006: incidence of hepatic tumors in Sprague-Dawley rats exposed to benzene by inhalation for 15 and 104 weeks.

| Group no. | Concentration, ppm | Schedule | Treatment | Animals | % of animals bearing tumors |
|-----------|-------------------|----------|-----------|---------|-----------------------------|
| I         | 200               | 4 hr/day, 5 days/week, 12 weeks | 7 weeks | B F 54 | 1.8 |
|           | 300               | 7 hr/day, 5 days/week, 85 weeks |         |         |                |
| II        | 200               | 4 hr/day, 5 days/week, 12 weeks | 7 weeks | E M 75  | 2.7 |
|           | 300               | 7 hr/day, 5 days/week, 85 weeks |         | F 45 | 10.8 |
| III       | 200               | 4 hr/day, 5 days/week, 12 weeks | 7 weeks | E M 140 | 6.4 |
|           | 300               | 7 hr/day, 5 days/week, 85 weeks |         |         |                |
| IV        | 0                 |          | Olive oil | B F 60 | 125 |
| V         | 0                 |          | (Controls) | E M 158 | 0.6 |

*B, breeders, E, embryos.

Experiment on Wistar Rats (Tables 26–28)

The most frequently expected tumors in this strain of rats, on the basis of the literature and of the historical controls of the BT Experimental Unit, are mammary tumors, leukemias, pheochromocytomas, and pheochromoblastomas. Moreover, a variety of other miscellaneous tumors were also observed (59).

The administration of benzene by ingestion is associated with an increase of total malignant tumors and carcinomas of Zymbal glands, oral cavity, and nasal cavities.

Table 26. Experiment BT 907: incidence of total tumors in Wistar rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

| Group no. | Dose, mg/kg | Sex | No. at start | NBMBA* | MTB* | No. of malignant tumors per 100 animals |
|-----------|-------------|-----|--------------|--------|------|----------------------------------------|
| 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   | M   | 40           | 75.0   | 20.0 | 25.0 |
|           | (Controls)  | F   | 40           | 85.0   | 25.0 | 30.0 |
|           |             | M+F | 80           | 80.0   | 22.5 | 27.5 |

*Total benign and malignant tumors.

Table 27. Experiment BT 907: incidence of mammary tumors, leukemias, pheochromocytomas and pheochromoblastomas in Wistar rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

| Group no. | Dose, mg/kg | Animals | Mammary tumors | % of animals bearing tumors |
|-----------|-------------|---------|----------------|----------------------------|
| I         | 500         | M 40    | BMT* | MT* | Leukemias | Pheochromocytomas | Pheochromoblastomas |
|           |             | F 40    | 2.5  | 5.0 | 2.5          | 2.5          |   |
|           |             | M+F 80  | 42.5 | 5.0 | 10.0         | 7.5          |   |
| II        | Olive oil   | M 40    | 22.5 | 2.5 | 7.5          | 1.2          |   |
|           | (Controls)  | F 40    | 10.0 | 2.5 | 5.0          | 2.5          |   |
|           |             | M+F 80  | 55.0 | 7.5 | 7.5          | 5.0          |   |

*Benign and malignant tumors.

*Malignant tumors (carcinomas).
Experiment on Swiss Mice (Tables 29–33)

The most frequently expected tumors in this strain of mice, on the basis of the literature and of the historical controls of the BT Experimental Unit, are mammary carcinomas (in females), lung tumors, leukemias, and hepatomas. Moreover, a variety of other miscellaneous tumors were also observed (59).

The administration of benzene by ingestion is associated with an increase of total malignant tumors, carcinomas of the mammary glands, lung tumors (adenomas, adenomas in deviation, and adenocarcinomas), and carcinomas of the Zymbal glands (together with an increase of dysplasias).

Table 29. Experiment BT 908: incidence of total tumors in Swiss mice exposed to benzene by ingestion (stomach tube) for 78 weeks.

| Group no. | Dose, mg/kg | Animals | Sex  | No. at start | No. of animals bearing tumors |
|-----------|-------------|---------|------|--------------|------------------------------|
|           |             |         |      |              | TBMT" | MT" | No. of malignant tumors per 100 animals |
| 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   | M       | 40   |              | 37.5  | 22.5 | 22.5 |
| (Controls)|             | F       | 40   |              | 40.0  | 27.5 | 27.5 |
|           |             | M+F     | 80   |              | 38.7  | 25.0 | 25.0 |

*Total benign and malignant tumors.
"Malignant tumors.

Table 30. Experiment BT 908: incidence of mammary carcinomas, pulmonary tumors, leukemias, and hepatomas in Swiss mice exposed to benzene by ingestion (stomach tube) for 78 weeks.

| Group no. | Dose, mg/kg | Animals | Sex  | No. at start | Mammary carcinomas | Pulmonary tumors | Leukemias | Hepatomas |
|-----------|-------------|---------|------|--------------|--------------------|-----------------|-----------|----------|
| I         | 500         | M       | 40   |              | 42.5               | 12.5            | 7.5       |
|           |             | F       | 40   |              | 47.5               | 37.5            | 20.0      |          |
|           |             | M+F     | 80   |              | 23.7               | 40.0            | 16.2      | 3.8      |
| II        | Olive oil   | M       | 40   |              | 2.5                | 7.5             | 12.5      | 5.0      |
| (Controls)|             | F       | 40   |              | 5.0                | 10.0            | 20.0      |          |
|           |             | M+F     | 80   |              | 3.7                | 8.7             | 16.2      | 2.5      |

Table 31. Experiment BT 908: incidence of pulmonary lesions of oncological interest in Swiss mice exposed to benzene by ingestion (stomach tube) for 78 weeks.

| Group no. | Dose, mg/kg | Animals | Sex  | No. at start | Total | Adenomatous hyperplasia-early adenomas | Adenomas | Adenomas* | Adenocarcinomas |
|-----------|-------------|---------|------|--------------|-------|----------------------------------------|----------|-----------|----------------|
| I         | 500         | M       | 40   | 42.5         | 2.5   | 22.5                                   | 15.0     | 2.5       |
|           |             | F       | 40   | 37.5         |  —    | 22.5                                   | 15.0     | 1.2       |
|           |             | M+F     | 80   | 40.0         | 1.2   | 22.5                                   | 15.0     | 1.2       |
| II        | Olive oil   | M       | 40   | 7.5          |  —    | 5.0                                    | 2.5      |          |
| (Controls)|             | F       | 40   | 10.0         |  —    | 10.0                                   |          |          |
|           |             | M+F     | 80   | 8.7          |  —    | 7.5                                    | 1.2      |          |

*Adenomas in deviation.
BENZENE: AN EXPERIMENTAL MULTIPOTENTIAL CARCINOGEN

Table 32. Experiment BT 908: incidence of Zymbal gland carcinomas and correlated precancerous lesions in Swiss mice exposed to benzene by ingestion (stomach tube) for 78 weeks.

| Group no. | Dose, mg/kg | Sex | No. at start | % of animals bearing lesions |
|-----------|-------------|-----|--------------|----------------------------|
|           |             |     |              | 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   | M   | 40           | -     | -         | -          |
|           | (Controls)  | F   | 40           | -     | -         | -          |
|           |             | M+F | 80           | -     | -         | -          |

Table 33. Experiment BT 908: incidence of hepatic tumors in Swiss mice exposed to benzene by ingestion (stomach tube) for 78 weeks.

| Group no. | Dose, mg/kg | Sex | No. at start | % of animals bearing hepatic tumors |
|-----------|-------------|-----|--------------|------------------------------------|
| I         | 500         | M   | 40           | 7.5                                |
|           |             | F   | 40           | 3.7                                |
|           |             | M+F | 80           | 3.7                                |
| II        | Olive oil   | M   | 40           | 5.0                                |
|           | (Controls)  | F   | 40           | -                                  |
|           |             | M+F | 80           | -                                  |

Experiment on RF/J Mice (Tables 34–37)

The most frequently expected tumors of this strain of mice, on the basis of the literature and of our experimental experience, are mammary carcinomas, pulmonary tumors, and leukemias. The most frequent histotype of hemolymphoreticular malignant neoplasias are lymphoblastic lymphosarcomas (much greater percentage) and lymphoblastic lymphosarcomas with histocytic component.

The administration of benzene by ingestion is associated with an increase of total malignant tumors, mammary carcinomas, lung tumors (adenomas, adenomas in deviation, and adenocarcinomas), and leukemias. In the treated animals the number of pulmonary tumors per tumor-bearing animal is greatly enhanced (Table 37).

Table 34. Experiment BT 909: incidence of total tumors in RF/J mice exposed to benzene by ingestion (stomach tube) for 52 weeks.

| Group no. | Dose, mg/kg | Sex | No. at start | No of malignant tumors per 100 animals |
|-----------|-------------|-----|--------------|--------------------------------------|
| I         | 500         | M   | 45           | 73.3                                 |
|           |             | F   | 40           | 85.0                                 |
|           |             | M+F | 85           | 78.8                                 |
| II        | Olive oil   | M   | 45           | 40.0                                 |
|           | (Controls)  | F   | 40           | 50.0                                 |
|           |             | M+F | 85           | 44.7                                 |

*aTotal benign and malignant tumors.
*bMalignant tumors.

Table 35. Experiment BT 909: incidence of mammary carcinomas, pulmonary tumors, leukemias, and hepatomas in RF/J mice exposed to benzene by ingestion (stomach tube) for 52 weeks.

| Group no. | Dose, mg/kg | Sex | No. at start | Mammary carcinomas | Pulmonary tumors | Leukemias | Hepatomas |
|-----------|-------------|-----|--------------|-------------------|------------------|-----------|----------|
| I         | 500         | M   | 45           | -                 | 51.1             | 57.8      | -        |
|           |             | F   | 40           | 22.5              | 45.0             | 60.0      | -        |
|           |             | M+F | 85           | 10.6              | 48.2             | 58.8      | -        |
| II        | Olive oil   | M   | 45           | -                 | 11.1             | 37.8      | -        |
|           | (Controls)  | F   | 40           | 2.5               | 7.5              | 35.0      | -        |
|           |             | M+F | 85           | 1.2               | 9.4              | 36.5      | -        |
Table 36. Experiment BT 909: incidence of pulmonary lesions of oncological interest in RF/J mice exposed to benzene by ingestion (stomach tube) for 52 weeks.

| Group no. | Dose, mg/kg | Sex | No. at start | Animals | Simple adenomatous hyperplasia | % of animals bearing lesions* |
|-----------|-------------|-----|--------------|---------|-------------------------------|-------------------------------|
| I         | 500         | M   | 45           |         | 51.1                          | Adenomas A                  |
|           |             | F   | 40           |         | 45.0                          | Adenomas A                  |
|           |             | M+F | 85           |         | 2.4                           | Adenomas A                  |
| II        | Olive oil   | M   | 45           |         | 2.2                           | Adenomas A                  |
|           |             | F   | 40           |         | 11.1                          | Adenomas A                  |
|           |             | M+F | 85           |         | 2.2                           | Adenomas A                  |

*For each tumor only the gravest lesion was counted.

Table 37. Experiment BT 909: incidence of total pulmonary lesions of oncological interest in RF/J mice exposed to benzene by ingestion (stomach tube) for 52 weeks.

| Group no. | Dose, mg/kg | Sex | No. at start | Animals | Simple adenomatous hyperplasia | No. of pulmonary lesions/100 animals* |
|-----------|-------------|-----|--------------|---------|-------------------------------|-------------------------------------|
| I         | 500         | M   | 45           |         | 24.4                          | Carcinomas of Zymbal gland          |
|           |             | F   | 40           |         | 17.5                          | Carcinomas of oral cavity          |
|           |             | M+F | 85           |         | 21.2                          | Carcinomas of nasal cavities       |
| II        | Olive oil   | M   | 45           |         | 2.2                           | Carcinomas of the skin             |
|           |             | F   | 40           |         | 10.0                          | Carcinomas of the forestomach      |
|           |             | M+F | 85           |         | 1.2                           | Carcinomas of the mammary gland    |

*All different lesions present in each animal were counted.

Multipotential Carcinogenicity, Dose-Response, and Effect of Age

A variety of tumors is associated to benzene exposure in the animals of all tested species and strains (Table 38).
A dose-response relationship was seen in the experiment with Sprague-Dawley rats. This relation appears particularly marked in the case of carcinomas of Zymbal glands, oral cavity, and nasal cavities when considered either singularly or together (Table 39).
An enhanced carcinogenic effect of benzene was observed in animals on which treatment was started during embryonal life (Table 24-28).

Table 38. Tumor associated to benzene exposure on the basis of the BT experimental project.

| Tumors                                           | Sprague-Dawley rat, ingestion | Inhalation | Wistar rat, ingestion | Swiss mouse, ingestion | RF/J mouse, ingestion |
|--------------------------------------------------|-------------------------------|------------|-----------------------|------------------------|-----------------------|
| Total 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 gland                  | (+)                           | (+)        | +                     | +                      | +                     |
| Hepatomas                                         | (+)                           | (+)        | +                     | +                      | +                     |
| Angiosarcomas of the liver                       | +                             | +          | +                     | +                      | +                     |
| Hemolymphoreticular neoplasias                   | (+)                           | (+)        | +                     | +                      | +                     |
| Tumors of the lung                               | +                             | +          | +                     | +                      | +                     |

*Weak evidence.
**Conclusions**

The experiments performed at the Bologna Institute of Oncology on benzene carcinogenesis have shown that benzene is a strong carcinogen on experimental animals; it is carcinogenic on four different types of experimental animals, i.e., Sprague-Dawley and Wistar rats, and Swiss and RF/J mice. Exposure to benzene is associated with an enhanced incidence of a variety of tumors; therefore, benzene must be considered a multipotential carcinogen. The neoplastic response associated with benzene exposure varies in the different types of tested animals. Benzene has carcinogenic effects when given both by inhalation and by ingestion. The carcinogenic effects of benzene increase by increasing the doses (daily dose, length of treatment). There is a high response when treatment is started during embryonal life.

The experimental research may still improve our knowledge of benzene carcinogenicity and correlated problems. At present, in our opinion, the following experimental research deserves full priority: a) studies on the carcinogenic effects of minimal doses of benzene (in the range of the present allowable levels), delivered by inhalation, to large groups of animals (megaexperiments); b) carcinogenicity studies on chemical mixtures containing benzene (fuels); c) carcinogenicity studies on chemically correlated and/or alternative compounds, i.e., toluene, xylenes, trimethylbenzenes, ethylbenzene, etc. Studies on these three fields of research are now ongoing or planned at the Bologna Institute of Oncology. Moreover, comprehensive epidemiological investigations on population groups exposed to benzene extended to all types of malignancies, with particular regard to lung carcinomas (on the basis of our recent experimental results) must be undertaken without delay.

This work was supported in part by EEC contract 323-79-4-ENV-I.

---

**REFERENCES**

1. Merian, E., and Zander, M. Volatile aromatics. In: Handbook of Environmental Chemistry, Vol. 3, Part B, Anthroprogenic Compounds (O. Hutzinger, Ed.), Springer Verlag, Berlin, 1982, pp. 117-161.
2. Howard, P. H., and Durkin, P. R. Sources of Contamination, Ambient Levels and Fate of Benzene in the Environment. EPA 500/5-75-006, U.S. Environmental Protection Agency, Washington DC, 1974.
3. Lanwerys, R. Benzene. In: Human Biological Monitoring of Industrial Chemical Series (L. Alessio, A. Berlin, R. Roi, and M. Boni, Eds.), Commission of the European Communities, Joint Research Centre, Ispra Establishment, 1983, pp. 2-22.
4. Brief, R. S., Lynch, J., Bernath, T., and Scala, R. A. Benzene in the workplace. Am. Ind. Hyg. Assoc. J. 41: 616-623 (1980).
5. Delore, P., and Borgomano, C. Leucémie aigüe au cours de l'intoxication benzénique. Sur l'origine toxique de certaines leucémies aigües et leurs relations avec les anémies graves. J. Med. Lyon 9: 227-233 (1928).
6. Vigliani, E. C. Leukemia associated with benzene exposure. In: Occupational Carcinogenesis. Ann. N.Y. Acad. Sci. 271: 143-151 (1976).
7. Goldstein, B. D. Hematotoxicity in humans. J. Toxicol. Environ. Health (suppl.) 2: 69-105 (1977).
8. Vigliani, E. C., and Saita, G. Benzene and leukemia. N. Engl. J. Med. 271: 872-876 (1964).
9. Goguel, A., Cavigneaux, A., and Bernard, J. Les leucémies benzéniqques de la région parisienne entre 1950 et 1965 (Etude de 50 observations). Nouv. Rev. Fr. Hematol. 7: 465-480 (1967).
10. Girard, R., Rigaut, P., Bertholon, J., Tolot, F., and Bourret, J. Les expositions benzéniques mérémées. Leur recherche systématique au cours des hémopathies graves. Enquête chez 200 hémopathes hospitalisés. Arch. Mal. Prof. Med. Trav. Secur. Soc. 29: 723-726 (1968).
11. Girard, R., Tolot, F., and Bourret, J. Hydrocarbures benzéniques et hémopathies graves. Arch. Mal. Prof. Med. Trav. Secur. Soc. 31: 625-636 (1970).
12. Girard, R., Prost, G., and Tolot, F. Comments on indemnification for benzene induced leukemia and aplasia. Arch. Mal. Prof. Med. Trav. Secur. Soc. 32: 581-583 (1971).
13. Girard, R., Tolot, F., and Bourret, J. Malignant hémopathies and benzene poisoning. Med. Lav. 62: 71-76 (1971).
14. Girard, R., and Revol, L. La fréquence d'une exposition benzénique au cours des hémopathies graves. Nouv. Rev. Fr. Hématol. 16: 477-484 (1970).
15. Aksoy, M., Dincol, K., Erdem, S., and Dincol, G. Acute leukemia due to chronic exposure to benzene. Am. J. Med. 52: 160-166 (1972).

16. Aksoy, M., Erdem, S., and Dincol, G. Leukemia in shoe workers exposed chronically to benzene. Blood 44: 837-841 (1974).

17. Ishimaru, T., Okada, H., Torniyasu, T., Tsuchimoto, T., Hoshino, T., and Ishimaru, M. Occupational factors in the epidemiology of leukemia in Hiroshima and Nagasaki. Am J. Epidemiol. 93: 157-165 (1971).

18. Pollini, G., and Colombi, R. Damage to bone-marrow chromosomes in benzene aplastic anaemia. Med. Lav. 55: 241-255 (1964).

19. Pollini, G., and Colombi, R. Chromosomal damage in lymphocytes during benzene haempathy. Med. Lav. 55: 641-655 (1964).

20. Forni, A. Chromosome changes due to chronic exposure to benzene. In: Proceedings of the International Congress of Occupational Health, Vienna, October 1966. Weiner Medizinische Akademie, Vol. 2/1, 1966, pp. 437-439.

21. Pollini, G., Biscardi, G. P., and Robustelli della Cuna, G. Chromosome changes in lymphocytes five years after benzene haemopathy. Med. Lav. 60: 743-758 (1969).

22. Forni, A., Cappellini, A., Pacifico, E., and Vigliani, E. C. Chromosome changes and their evolution in subjects with past exposure to benzene. Arch. Environ. Health 28: 385-391 (1971).

23. Forni, A., Pacifico, E., and Limonta, A. Chromosome studies in workers exposed to benzene or toluene or both. Arch. Environ. Health 22: 373-378 (1971).

24. Khan, H., and Khan, M. H. Cytogenetic studies following chronic exposure to benzene. Arch. Toxicol. 31: 39-49 (1973).

25. Girard, R., Malein, M. L., Berthöl, J., Ceurar, P., and Evreux, J. Étude de la phosphatase alcaline leucocytaire et du cytype des ouvriers exposés au benzène. Arch. Mal. Prof. Med. Trav. Secur. Soc. 31: 31-38 (1970).

26. Hartwig, G., and Schwitz, G. Chromosome studies after chronic exposure to benzol. Dutch. Med. Wochenschr. 97: 45-49 (1972).

27. Fredga, K., Reitalu, J., and Berlin, M. Chromosome studies in workers exposed to benzene. In: Genetic Damage in Man Caused by Environmental Agents (E. K. Betz, Ed.), Academic Press, New York, 1979, pp. 187-203.

28. Lügnac, G. O. E. Die Benzangleukämie bei Menschen und weissen Mausen. III. Zweite Benzoelversuchsreihe von 54 Mausen geben 8 an leukämie oder Lymphoblastoma infiltrus alvearemizum zugrunde-fürtere Teerverbenzolsuche. Krankheitsforsch. 9: 426-433 (1932).

29. Kirshbaum, A., and Strong, L. C. Influence of carcinogens on the age incidence of leukemia in the high leukemia F strain of mice. Cancer Res. 2: 841-845 (1942).

30. Amiel, J. L. Essai néglatif d’induction de leucémies chez les souris par le benzène. Rev. Fr. Etud. Clin. Biol. 5: 198-199 (1960).

31. Hiraki, K., Irino, S., and Miyoshi, I. Development of subcutaneous sarcomas in Swiss mice given repeated injections of benzene in olive oil. Gann 54: 427-431 (1963).

32. IARC. Some anti-thyroid and related substances, nitrofurans and industrial chemicals. IARC Monograph on the Carcinogenic Risk of Chemical to Man, Vol. 7. International Agency for Research on Cancer, Lyon, France, 1974.

33. Maltoni, C., and Scarnato, C. Le prime prove sperimentali del l’azione cancerogena del benzene. Gli Ospedali della Vita 4: 111-113 (1977).

34. Maltoni, C., and Scarnato, C. First experimental demonstration of the carcinogenic effects of benzene. Long-term bioassays on Sprague-Dawley rats by oral administration. Med. Lav. 70: 352-357 (1979).

35. Maltoni, C., Conti, B., and Scarnato, C. Squamous cell carcinomas of the oral cavity in Sprague-Dawley rats, following exposure to benzene by ingestion. First experimental demonstration. Med. Lav. 73: 441-445 (1982).

36. Maltoni, C., Cotti, G., Valgimigli, L., and Mandrioli, A. Zymbal gland carcinomas in rats following exposure to benzene by inhalation. Am. J. Ind. Med. 3: 11-16 (1982).

37. Maltoni, C., Cotti, G., Valgimigli, L., and Mandrioli, A. Hepatocarcinomas in Sprague-Dawley rats, following exposure to benzene by inhalation. First experimental demonstration. Med. Lav. 73: 446-450 (1982).