

The First Experimental Proof of the Carcinogenic Action
of Benzene

Cesare Maltoni and Corrado Scarnato
Institute of Oncology and Center of Tumor
Bologna, Italy

Benzene has the following physical

chemical characteristics:

Molecular weight	78.1
Description	clear liquid, colorless, highly flammable
Boiling point	80.1°C
Solidifies at	5.5°C
Density	d ₁₅ 0.8787
Refractive Index	n _D ²⁰ 1.5016
Volatility	vapor pressure is 74.6 mm Hg at 20°C
Solubility	weakly soluble in water, missible with acetone, alcohol, carbon disulfide, carbon tetrachloride, chloroform, ether, glacial acetic acid and oils

Presently, in progress in the USA, there is a revision of the standard of exposure with a proposal of a TLV of 1 ppm and 0.1 ppm.

Prolonged exposure to benzene causes toxic effects in the marrow, both in men and in experimental animals.

As early as 1928, the association between chronic exposure to benzene and the occurrence of leukemia was proposed. Since then, in many countries among them Italy, numerous cases of occupational leukemia in workers exposed to benzene have been reported in the literature. The majority of leukemia cases from benzene have been verified in Lombardy, Emilia - Romagna and Tuscany among workers of the shoe industry and the clothing industry. Generally, the cases of leukemia from benzene, described in the literature were verified in patients with a preceding history of medullary poisoning.

Despite the importance of the compound, the number of individuals potentially exposed and despite the record of cases of occupational leukemia, adequate epidemiological investigation and experimental research were not available. In 1974, 46 years after the first sign of an association between exposure to benzene and leukemia, upon examining the experimental tests of carcinogenicity, one could conclude that: Benzene was tested only in rats by subcutaneous injection and by cutaneous application. The reported data did not permit one to conclude that carcinogenic activity was demonstrated (IARC Monograph, Vol. 7). One might add, it did not demonstrate the absolute inadequacy of the experiments.

In conclusion, at the time in which we began the present research, the suspicion of the carcinogenicity of benzene, lacking epidemiological tests and adequate experimental research, was based on the recorded cases of association between benzene poisoning and leukemia, leaving room for uncertainty and ambiguity, sometimes malicious, and/or on the hypothesis that the eventual cancer effect manifested itself only at toxic doses.

Benzene is produced commercially from coal and petroleum. Presently the largest source of benzene is petroleum. It is one of the major compounds produced. The world production runs over 15 million tons a year. The major producers are the U.S., Western Europe and Japan. Italy produces over $\frac{1}{2}$ million tons.

The compound was used, especially in the past, in the production of commercial gasoline. Presently benzene is used almost entirely as an intermediate chemical for the production of ethylbenzene, phenol, cyclohexane, malein anhydride, aniline, dichlorobenzene, etc., which at this time affect numerous industries particularly those of plastics, resins, elasomers and dyes. Benzene has been used as a solvent for paints, rubbers and adhesives and also been used in medicine in the treatment of hemoblastomas (leukemia, polycythemia vera and malignant lymphoma) and in veterinary science for the disinfection of wounds.

Those potentially exposed to benzene are:

1. Workers employed in the production of benzene;
2. Workers who use the compound, as such, for various industrial uses;
3. Workers who use compounds which contain benzene as a component (gasoline) as a solvent (for example adhesives) or as an impurity (for example industrial toluene);
4. Those who live in the vicinity of factories which produce benzene or factories or works which use it.

Because of its high volatility, the risk of exposure to benzene from these groups exceeds the amount in which it is present in gasoline.

The permissible levels of benzene in the work environment vary from country to country. In the USA the TWA in 8 hours is 10 ppm with a "ceiling" of 25 ppm, except for 10 minutes in which the concentration may reach 50 ppm; in other countries the maximum level allowable is 25 ppm; in the USSR the maximum permissible level is 6 ppm.

Among other things, there is also this very important aspect, even though in our opinion the lack of experimental verification of the carcinogenicity of benzene is due to the inadequacy of the experiments, the pretext of skepticism concerning the predictive potentiality of long term tests of carcinogenicity has constituted, on the whole, an analogous situation related to arsenic.

To verify the potential carcinogenicity of benzene and with the intent of clarifying once again the validity of predictive tests, in April, 1976 we initiated an experiment for testing the long term effects of benzene in a rat. In this experiment benzene, dissolved in olive oil, was administered to Sprague Dawley rats, 13 weeks old, by means of a gastric probe, 4-5 times a week for 52 weeks at a dose of 250 and 50 mg/kg of body weight. The control rats were treated only with olive oil.

The animals were kept alive until natural death occurred and during and after the treatment were checked and weighed every 15 days. All the removable macroscopic lesions were recorded. A complete autopsy was performed on all the animals that died and histological examinations were conducted on the Zymbal gland, the retrobulbary gland, interscapular dark fat, salivary gland, tongue, lungs, liver, kidneys, spleen, stomach, diverse segments of the intestine, bladder, encephalon and any other organ with pathological lesions.

The experimental plan and the results are shown in Table 1. The treatment with benzene, in our experimental conditions, did not establish toxic effects of particular importance (relevancy).

The occurrence in the treated groups of Zymbal gland carcinomas and of dermatofibromata, which were not observed in the control group and which are of rarest encounter in the strain of rat used, clearly gives evidence of the carcinogenicity of benzene. Such a conclusion is supported also by the major concentration of other tumors spontaneously occurring which are not common in

the group treated with the maximum dose and by the short period of latency in the occurrence of mammary tumors in the treated animals.

In the carcinogenic response, the diverse distribution in the two sexes of carcinoma of the Zymbal gland and of cutaneous dermatofibromata is interesting from the point of view of the effect of the action related to the animals.

The data displayed here confirms the carcinogenicity of benzene. In addition it demonstrates that benzene provokes substantial tumors at doses which do not provoke important toxic effects. Meanwhile it establishes a further verification of the reliability of the predictive tests in oncology.

On the basis of our results, the epidemiological investigation of the risk to the group which is inflicted over a time is not only to leukemia but to all tumors. We have presented these results, without waiting for the end of the study (which is scheduled for not later than the end of 1978), in consideration of the fact that presently they are debating in various regions the levels of exposure (to benzene) in the work environment.