

Technical Report

No 16

**A review of recent literature
on the Toxicology of
Benzene**

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A REVIEW OF RECENT LITERATURE ON
THE TOXICOLOGY OF BENZENE

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A. SUMMARY

The Health and Safety Executive (1982) in the UK and the Conseil Européen des Fédérations de l'Industrie Chimique (CEFIC, 1983) have recently reviewed the toxicology of benzene. Since these reports were issued a number of publications have appeared which need to be assessed. Such an assessment is made by ECETOC in this Technical Report.

Papers on the short- and long-term toxicity of benzene, comprising studies of the exposure of rats and mice by gavage and inhalation, are reviewed. It is concluded that benzene is a carcinogen to rats and mice under the conditions of the experiments.

Numerous recent publications on the induction of chromosomal damage by benzene in tissues from rats and mice (in vivo), and chinese hamsters and human lymphocytes (in vitro), confirm that benzene is clastogenic and suggest that at least part of its activity may be due to its metabolites. Recent cytogenetic studies of lymphocytes from workers occupationally exposed to benzene showed no consistently significant elevation in chromosomal aberrations which can be related directly to such exposure. No relationship between the types of chromosome damage observed and effects of exposure to benzene on human health can be established at present.

Several epidemiological studies have appeared recently. The Task Force notes that while there is adequate evidence that exposure to high concentrations of benzene is associated with haematotoxicity and acute myelocytic leukaemia in humans, the question of whether benzene can cause an increased incidence of other forms of lymphopoietic or haematopoietic cancers remains unresolved. It is likely to remain unresolved under the conditions of modern benzene-using operations where the numbers exposed, and the degree of exposure, are low.

Such reports as have been published on the effects of exposure to benzene on human reproduction are contradictory and inconclusive. There is no reliable evidence to suggest any association between exposure to benzene and adverse effects on human reproduction.

The papers published so far on the consequences for human health of exposure to benzene are, without exception, deficient in quantitative exposure data.

Without such data it is impossible to describe the shape of the dose-response curve relating exposure with any of the recorded toxic effects. If future epidemiological studies are to increase our understanding of the adverse effects of benzene on human health they must meet the requirements specified in this report. Little is to be gained in continuing to report the mortality or morbidity of populations exposed to benzene under inadequately-defined conditions.

The recent studies reviewed in this report contribute no new medical or toxicological evidence which justifies amending current benzene exposure standards.

B. INTRODUCTION

Benzene is an important, large-tonnage industrial chemical to which a substantial number of people are exposed. The toxicology of benzene has been reviewed recently by the UK Health and Safety Executive (1982) and CEFIC (1983) in documents which cover the literature up to about 1980 or 1981. Since these reviews were published a number of papers on the animal toxicology of benzene and its effects on human health have appeared, and they are assessed in this report.

In general, this review is restricted to publications which are not dealt with in the above two documents, but some reference back to earlier papers was occasionally necessary.

C. SHORT AND LONG TERM ANIMAL TOXICOLOGY

(The nomenclature for the variety of blood disorders, including various forms of leukaemia, mentioned in this report is somewhat complex, and synonyms are sometimes used for the same disease. Therefore, the terminology of the cited authors is used in the text and a Glossary of Terms has been included in Appendix 2).

This section deals mainly with papers which are most relevant to human exposure and current exposure standards, i.e. to studies on the effects of benzene on experimental animals exposed by inhalation. Some studies on oral exposure are, however, also discussed.

1. Short Term Toxicology

In inhalation studies on immunofunction and haematotoxicity, Baarson et al. (1984) exposed male C57B1 mice to 10 ppm of benzene, 6h/d, 5d/w, for up to 178 days, and Rozen et al. (1984) exposed mice of the same strain and sex to 10, 30, 100 and 300 ppm for 6h/d, for 6 days. The authors concluded that benzene in concentrations around the current occupational exposure limit (10 ppm) may have an influence on immunofunction and may be haematotoxic. This conclusion is based on i) the observed depression in the number of splenic nucleated erythrocytes, and of circulating erythrocytes and lymphocytes, and ii) the depression of the in vitro colony-forming ability of erythroid progenitor cells and depression of in vitro colony mitogen-induced blastogenesis of femoral B- and splenic T-lymphocytes.

Detailed data, eg. numbers of femoral erythroid cells, colony-forming ability of femoral T-lymphocytes and splenic B-lymphocytes, and the differential count of the lymphocyte population, are not given in the papers. There is no critical analysis of such important factors as dose-reponse relationships, trends with time, statistical significance or any limitations of the techniques used. This makes the evaluation of the results difficult. Some of the numerical data vary over a wide range, and it seems questionable whether these results could be reproduced. There is little experience of the biological relevance of certain of the changes in immune cell functions as detected by the techniques used, and these techniques need to be validated by studying a range of chemicals.

In a study for the American Petroleum Institute (Hazelton Labs., 1983), male and female CD-1 mice and SD-CD rats were exposed, by inhalation, to 0, 1, 10, 30 and 300 ppm of benzene, 6 h/d, 5 d/w for 90 days. At 300 ppm, testicular, ovarian and bone marrow damage were found in mice. Gonadal damage included testicular atrophy and degeneration, with a decrease in the number of spermatozoa in the epididymal ducts and an increase in the incidence of abnormal sperm forms. Four out of forty females had bilateral ovarian cysts. At 1, 10 and 30 ppm, only minimal testicular and ovarian effects of doubtful biological significance were observed, in the mice only.

Exposure of rats to 300 ppm resulted in lower body weights, decreased total leukocyte and lymphocyte counts, and increased neutrophil counts. A tendency

towards decreased cellularity in the femoral bone marrow was seen in the same group. At lower doses no treatment-related effects were observed in rats.

Toft et al.(1982) found that when NMRI mice were exposed either continuously during 4-10 days, or intermittently (8 h/d, 5 d/w for 2 weeks), to concentrations of benzene in air ranging from 1 to 200 ppm, haematotoxic effects occurred at 20 ppm. The effects were on nucleated red cells, and the colony-forming granulopoietic stem cells, of the tibia.

2. Long Term Toxicology

2.1. Oral exposure

2.1.1. Maltoni et al.(1982a, 1982d, 1983). These reports relate to a single study in which a dose of 500 mg/kgbw of benzene dissolved in olive oil was administered to 40 male and 40 female Sprague-Dawley rats, by stomach tube, once per day on 4 or 5 d/w. The study was intended to run for 104 weeks. Control groups of 50 males and 50 females received olive oil only. The rats were 7 weeks old at the start of the study and were observed until they died spontaneously or had to be sacrificed in a moribund state. Gross observations and body weights were recorded, and some haematological examinations on circulating blood were performed on a limited number of rats at week 84. Standard autopsies and histopathology were carried out on sacrificed rats or on those found dead.

In the 1983 paper, results are reported at 92 weeks. Exposure to benzene correlated with slightly lowered body weights in both sexes, more marked in females than in males, and with reduced red and white blood cell counts which were mostly due to lymphocytopenia. Survival at week 92 was higher in the exposed males (72.5%) than in the controls (58%), but slightly lower for the females (60, against 66% in the controls). The incidence of Zymbal gland carcinomas was 15% in both exposed males and females, and one occurred in a male of the control group. This tumour has been reported to occur at a frequency of 0.9% in the strain of rat used (Maltoni and Scarnato, 1983).

Carcinomas of the oral cavity originating mainly at the lips were observed with incidences of 17.5 and 10% in males and females, respectively, and were zero in the controls and historical controls. One

carcinoma in the nasal cavity of a treated male was also reported. One unspecified type of leukaemia was observed in a female rat of the control group and in one of the dosed animals, but neither the bone marrow nor lymphnodes was examined. One angiosarcoma of the liver was observed in a female of the exposed group.

2.1.2. Maltoni and Scarnato (1979, 1983) reported a similar study on groups of 30 male and 30 female Sprague-Dawley rats (dose : 50 mg/kgbw) and 35 of each sex (dose : 250 mg/kgbw), with 30 animals of each sex in control groups receiving olive oil only. Treatment up to week 52 was followed by an observation period until the animals died. In this study no haematology, or histopathology of the lymphnodes or bone marrow, was reported. The tumour incidences at week 144 (termination of the study) are shown in Table 1.

TABLE 1
Incidence of Tumours in Sprague-Dawley Rats
in Study by Maltoni and Scarnato (1979, 1983)

	<u>Control</u>		<u>50 mg/kgbw</u>		<u>250 mg/kgbw</u>	
	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>
Zymbal Gland Carcinoma	0/28	0/30	0/28	2/30	0/33	8/32
Leukaemia	0/28	1/30(a)	0/28	2/30(b)	4/33(c)	1/32(d)
Carcinoma of oral cavity	0/28	0/30	0/28	0/30	0/33	2/32
Mammary Carcinoma	0/28	3/30	0/28	4/30	0/33	7/32
Angiosarcoma	0/28	0/30	0/28	0/30	1/33(e)	0/32
Hepatoma	0/28	0/30	0/28	0/30	1/33	0/32

- (a) haematosarcoma
- (b) 1 myelogenous leukaemia, 1 haematosarcoma
- (c) 1 lymphoblastic leukaemia, 3 haematosarcomas
- (d) 1 lymphocytic leukaemia
- (e) subcutaneous

Apart from the results reported above, the authors stated that benzene "did not show acute or sub-acute toxic effects". No data were provided on time-related mortality or time-of-occurrence of the tumours.

2.1.3. NTP study. Two-year toxicology and carcinogenicity studies with oral dosing have been performed on F344/N rats and B6C3F₁ mice under the US National Toxicology Programme (NTP, 1984).

- a) Rat study. Doses of 0, 50, 100 and 200 mg/kgbw of benzene in corn oil (5 ml/kg) were administered by gavage to groups of 50 male rats for 5 d/w over 103 weeks. Groups of 50 female rats were similarly administered 0, 25, 50 and 100 mg/kgbw. Ten additional animals were placed in each group for interim sacrifice at 12 months.

Daily observations on the health status of the animals were made. Haematological analyses (HCT, RBC, WBC, differential WBC, reticulocyte count, prothrombin time, haemaglobin and MCV) were performed on 10 animals from each sex and group at 12, 15, 18 and 21 months from the start of the study. These analyses were also performed on the additional animals selected for interim sacrifice after 0, 3, 6, 9 and 12 months exposure. Rats killed in extremis, found dead, or sacrificed at the end of the study were autopsied and detailed histopathological investigations were carried out.

The mean body weight of the male rats in the 200 mg/kgbw group at week 103 was 23% lower than that of the controls administered corn oil only. The survival in all exposed groups was lower than that of the controls, and was dose-dependent :

	Controls	25	50	100	200 mg/kgbw
males	32/50	-	29/50	25/50	16/50
females	46/50	38/50	34/50	25/50	-

Effects on the haematopoietic system, Zymbal gland, stomach, adrenals, skin and oral cavity were found. An increased incidence of lymphoid depletion of the splenic follicles was observed in males and females. Dose-related leukopenia occurred in both sexes during the first 18 months of the study. Zymbal gland carcinomas were found in the exposed groups of both sexes at a higher incidence than in the controls :

	Controls	25	50	100	200 mg/kgbw
males	2/50	-	6/50	10/50*	17/50*
females	0/50	5/50*	5/50*	14/50*	-

(Detailed statistical analyses by various methods are given in the NTP paper, and throughout this section * indicates a statistically-significant difference between the exposed and control animals as determined by the Fisher test, $p < 0.05$).

The incidence of epithelial hyperplasia of the Zymbal gland was also higher than that of the controls for the mid- and high-dose males, and high-dose females.

	Controls	25	50	100	200 mg/kgbw
males	0/49	-	3/48	12/50*	10/49*
females	1/49	1/45	2/50	6/49*	-

Exposure to benzene was associated with increased incidences of neoplasms of the skin (males only) and oral cavity. The incidences of squamous cell papillomas and squamous cell carcinomas of the skin were :

	Controls	50	100	200	mg/kg/bw
males	0/50	2/50	1/50	5/50	(papillomas
males	0/50	5/50	3/50	8/50*	carcinomas)

Squamous cell papillomas and squamous cell carcinomas of the oral cavity were observed in exposed rats at incidences which were statistically significantly higher than in the controls for both types, separately and combined. The incidences for both types combined were :

	Controls	25	50	100	200 mg/kgbw
males	1/50	-	9/50*	16/50*	19/50*
females	1/50	5/50*	12/50*	9/50*	-

- b) Mouse study. Doses of 0, 25, 50 and 100 mg/kgbw of benzene were administered to male and female mice by gavage, for 5d/w over 103 weeks, in a study conducted with a protocol similar to that used for rats. Mean body weights of the mice in the 100 mg/kgbw group were 14

and 17% less than those of the controls, for males and females respectively. There was no effect on body weight at lower levels. The survival of dosed animals was lower than that of the controls, and was dose-dependent, as shown by the mortality figures:

	Controls	25	50	100	mg/kgbw
males	7/50	18/50	23/50	28/50	
females	18/50	24/50	26/50	30/50	

Effects on the haematopoietic system, Zymbal gland, stomach and adrenals were found (as in rats), but there were no lesions of the oral cavity in the mice. The incidence of malignant lymphomas was higher than that in the controls for all treated groups :

	Controls	25	50	100	mg/kgbw
males	4/49	9/48*	9/50*	15/49*	
females	15/49	24/45*	24/50*	19/49*	

Bone marrow haematopoietic hyperplasia was observed at increased incidences in both sexes :

	Controls	25	50	100	mg/kgbw
males	0/49	11/48*	10/50*	25/49*	
females	3/49	14/45*	8/50*	13/49*	

Dose-related leukopenia was observed in both sexes in the first 18 months only. Zymbal gland carcinomas were of higher incidence than in the controls in the case of the mid- and high-dose male mice and the high-dose female mice :

	Controls	25	50	100	mg/kgbw
males	0/49	1/48	4/50*	21/49*	
females	0/49	0/45	1/50	3/49*	

In mid- and high-dose males, and high-dose females, the incidence of epithelial hyperplasia of the Zymbal gland was also increased :

	Controls	25	50	100	mg/kgbw
males	0/49	3/48	12/50*	10/49*	
females	1/49	1/45	2/50	6/49*	

In mice of both sexes there was a higher incidence of alveolar epithelial hyperplasia at 50 and 100 mg/kgbw, and alveolar/bronchiolar adenomas and carcinomas were observed at incidences higher than in the controls in the mid- and/or high-dose groups. Other lesions such as focal hyperplasia and adenomas of the Harderian gland, and hyperplasia and squamous cell carcinomas of the preputial gland, were also of higher incidence in the dosed mice. Mammary carcinomas and carcinosarcomas occurred at higher incidences in the mid- and/or high-dose groups. Hepatocellular adenomas and carcinomas were also of higher incidence in treated females than in the controls, and were dose-related :

	Controls	25	50	100	mg/kgbw
females	0/49	2/45	5/50*	10/49*	(adenomas)
females	0/49	0/45	1/50	4/49*	(carcinomas)

The authors concluded that there was clear evidence that benzene was carcinogenic to rats and mice.

2.2. Exposure by inhalation

2.2.1. Maltoni et al.(1982b, 1982c and 1983). In these three publications

a single study is reported in which 12-day pregnant Sprague-Dawley rats were exposed to benzene at 200 ppm, 4 h/d, for 5 d/w until parturition. The offspring were then exposed to 200 or 300 ppm of benzene as follows:

- a sub-group i) of 70 male and 59 female offspring was exposed to 200 ppm, 4 h/d, 5 d/w for 7 weeks, and then 7 h/d, 5 d/w for 8 weeks;
- a sub-group ii) of 75 male and 65 female offspring was exposed to 200 ppm, 4 h/d, 5 d/w for 7 weeks and then 7 h/d, 5 d/w for 12 weeks followed by an exposure to 300 ppm 7 h/d, 5 d/w for 85 weeks. The total exposure was for 104 weeks.

Groups of 158 males and 149 females were used as controls. After cessation of exposure, the animals were observed for the remainder of

their life. The latest publication (Maltoni et al., 1983) gives the results at week 118, at which time the study was still continuing.

The body weight of the exposed males was slightly affected. Mortality was higher than in the controls, but may be significant only in sub-group (ii). The WBCs tended to be lower in sub-group (ii) after 98 weeks of exposure. There were no exposure-related effects on WBCs or RBCs. The incidence of hepatic pre- and neo-plastic lesions were :

- controls ; no hepatomas, or nodular hyperplasia or dysplasia.
- sub-group (i) ; 3 hepatomas in females only, nodular hyperplasia in 2 males and 7 females.
- sub-group (ii) ; hepatomas in 2 males and 1 female, nodular hyperplasia in 1 male and 5 females, 2 nodular dysplasias in females only.

The incidence of leukaemias was :

- controls ; 1 in a male.
- sub-group (ii); 2 in males.

No Zymbal gland tumours were observed. The exposed parent females developed 1 hepatoma and 5 nodular hyperplasias, whereas none was observed in the control group.

2.2.2. Snyder et al.(1980) exposed groups of 50 AKR/J and 40 C57BL/6J male mice to benzene, by inhalation, at levels of 100 and 300 ppm respectively, for 6h/d, 5d/w for life. The same numbers of animals were used as controls. Clinical observations and body weight were regularly recorded, and haematological studies (RBC, and total and differential WBCs) were performed every second week on 10 exposed and 10 control mice. Gross pathology was carried out on all animals, and routine histopathology on the lung, spleen, liver, kidneys and bone marrow only.

Exposure to 100 ppm had no influence on the survival or body weight gain of the AKR/J strain, but a significant incidence of lymphocytopenia occurred at an early stage in the study. RBCs were also depressed, and bone marrow hyperplasia was of significantly higher incidence in the 100 ppm group (10, as against 1 in the controls). No other significant effect was reported for the AKR/J strain.

The body weight gain and survival of C57BL/6J mice exposed to 300 ppm of benzene were lower than in the controls. Lymphocytopenia, RBC depletion, and other haematological changes such as anisocytosis, poikilocytosis, and left shift in differential WBC also occurred in the exposed mice. Histological examination showed a higher incidence of haematopoietic neoplasms (controls 2/40; exposed animals 8/40) comprising 2 lymphomas in the controls and 6 in exposed animals, plus 1 myeloma and one leukaemia in the exposed. Bone marrow and spleen hyperplasia (non-neoplastic lesions) were of higher incidence in the exposed than in the control group, the spleen hyperplasia being due mainly to ectopic haematopoiesis.

- 2.2.3. Goldstein et al. (1982) exposed groups of 40 CD1 mice to 0 and 300 ppm of benzene, 6h/d, 5d/w for 31 weeks (see below), followed by observation for life. One of the mice exposed to 300 ppm developed a chronic myelogenous leukaemia, another an acute myeloblastic leukaemia, and a third had a granulocytic hyperplasia. Two other unspecified tumours were reported. Further details of the same study are given by Snyder et al. (1982) who found that life was shortened at 300 ppm, the exposure therefore being discontinued after 31 weeks. Five neoplasms (2 malignant lymphomas and 2 myeloid leukaemias already reported by Goldstein, plus a benign adenoma) were found in the 300 ppm group, and two neoplasms (malignant lymphomas with no thymic involvement) were found in the controls. Bone marrow hyper- and hypo-plasia, and an increase in splenic haemosiderin pigments, were observed in the 300 ppm group at a statistically-significant higher incidence.

Because the number of surviving animals was low, as was the number of neoplasms (there was no statistically-significant difference between the exposed and control groups) it is difficult to draw conclusions from this study, except that there were clear effects on the haematopoietic system at 300 ppm.

- 2.2.4. Snyder et al. (1984). Haematological investigations (RBC, and total and WBCs) were made several times within the first 1.5 year of the exposure of male Sprague-Dawley rats (40 controls and 40 exposed) to 100 ppm of benzene by inhalation. Animals found dead, or sacrificed in a moribund condition, were autopsied. Samples from lung, liver, spleen, bone

marrow, and kidney were investigated histologically, as were any abnormalities.

During the study, an epidemic respiratory infection occurred between days 448 and 504, with resulting high mortality (20 controls and 13 exposed rats died). No differences in weight gain between the groups were observed. Erythrocyte and lymphocyte counts of exposed rats were lower than those of the controls but were rarely significant. Eleven exposed rats developed neoplasms, including 1 chronic myelogenous leukaemia, 2 Zymbal gland carcinomas, 1 hepatoma, 1 cholangioma, 1 liver haemangioma, 1 liver haemangio-endothelioma, and 1 fibrosarcoma and squamous cell carcinoma in the facial area. Only 3 tumours (1 fibrosarcoma and one fibroma of the trunk, and 1 osteogenic sarcoma of the mandible) occurred in the control animals. The mortality-corrected incidences of both total and malignant tumours in the treated rats were not significantly greater than those in the controls.

The authors concluded from the limited data available, that 100 ppm of benzene was haematotoxic and carcinogenic to rats.

2.3. Discussion and conclusions

The above long-term studies on rats by Maltoni et al., most of which had not been completed at the time of writing, are lacking in a number of requirements which would permit a full evaluation of the results (important details are not given, and the results are not evaluated statistically). The unusual exposure regime in the Maltoni inhalation study also makes interpretation difficult. Although the haematopoietic system is involved in certain of the effects, no historical data are given. Only limited haematological data are reported but there is no detailed description of the methods by which they were determined. Data on bone marrow and lymphnodes are also lacking. The background incidence of tumours is considered in a few cases, but fluctuations in this incidence rather than a single percentage value are important for assessing the results of a specific study.

Regarding Zymbal gland tumours, although the data of Maltoni et al. may reflect only normal fluctuations rather than benzene-related tumours, it is significant that other investigators also reported a higher incidence

of these. The significance of the hepatic tumours found by Maltoni et al. is difficult to assess because no criteria for classifying them are given, and there is no correlation of the incidence of hepatomas with the duration of exposure in the 2 groups. No other study indicated a dose-related increase in the incidence of this type of tumour.

The occurrence of tumours in the oral cavity has been demonstrated by both Maltoni and the NTP. These tumours may have arisen by local contact during oral administration, and their relevance to the exposure of man by inhalation is uncertain.

The oral long-term studies can be interpreted only with great care when the results are extrapolated to assess exposure by inhalation since massive dosing by gavage leads to excessively-high peak blood levels not reached on exposure by inhalation.

The excess incidence of leukaemias in the Maltoni oral studies seems not to reflect a trend since at the highest dose studied (500 mg/kgbw) only one was reported, which corresponds to his historical control data. At the lower doses either no lesions are reported or, if they are present, their incidence is low and no histological details are given. In the Snyder et al.(1980) study, haematopoietic neoplasms were induced in one strain of mice exposed to 300 ppm of benzene by inhalation. The tumours were mostly lymphomas, and there was only one leukaemia. The second strain of mice, exposed to 100 ppm, did not develop a significantly higher incidence of haematopoietic tumours.

In summary, while none of the Maltoni studies on its own may be considered as proof of the carcinogenic action of benzene, when they are taken together and considered along with the NTP rat and mouse study and the Snyder study on mice it is clear that higher incidences of tumours have occurred, in some cases in a dose-related manner. The Task Force concluded that overall there is adequate evidence for the carcinogenic action of benzene on rats and mice under experimental conditions, and that benzene must therefore be categorised as an animal carcinogen. The Task Force notes that the types of neoplasm (hepatomas, oral carcinomas) reported in the recent animal studies are rare in humans. In view of the long period over which workers exposed to benzene have been under observation, the

Task Force believes that if a causal association existed between human exposure to benzene and the onset of such neoplasms, it would have been detected. Benzene is carcinogenic to rats, mice and man, but the substantial differences in the responses makes the qualitative and quantitative extrapolation of the animal results to man uncertain.

D. MUTAGENICITY AND CLASTOGENICITY

1. Experimental Data

1.1. Mutagenicity. It is well established that benzene does not induce point-mutations in chromosomes (Styles and Richardson, 1984). Recent papers on the Salmonella reverse-mutation assay (Seixas et al., 1982) and the DNA-repair assay on E. Coli (de Flora et al., 1984) confirm this.

1.1.1. Chromosome damage in vitro. It has proved difficult in the past to obtain a positive clastogenic response in cells exposed to benzene in vitro. However, as the auxiliary metabolic activation systems employed in these assays have become better developed a number of positive results have been reported.

- a) Human lymphocytes treated in vitro. Tanaka Ryuji (1981) reported that benzene produced various structural chromosomal aberrations following the in vitro treatment of human lymphocytes. This observation has since been confirmed by Howard et al. (1984) who found that structural chromosomal aberrations were induced at dose levels as low as 9 µg/ml in the blood from a male, and 44 µg/ml in the blood of a female.
- b) Sister chromatid exchange in human lymphocytes. Morimoto and Wolff (1980) found no increases in the frequency of SCEs due to benzene, but found substantial increases due to its principal metabolites, catechol and hydroquinone. However, it has been subsequently reported by Morimoto (1983) that, following optimisation of the auxiliary metabolic activation system, benzene itself induces SCE at dose levels as low as 2×10^{-4} M. The results were confirmed for benzene and enhanced positive results were reported with catechol, hydroquinone and phenol (Morimoto et al., 1983).

- c) Chinese hamster cells. Palitti (1984) reported positive results with Chinese hamster ovary cells exposed to benzene, the lowest effect-concentration being 100 µg/ml. Ishidate (1984) also found positive effects in Chinese hamster lung cells at a dose of 1,100 µg/ml. Benzene produces significant levels of aneuploidy (random chromosomal loss or gain) in Chinese hamster liver cells (Danford, 1984).

1.1.2. Chromosome damage in vivo

- a) Mouse micronucleus test. Increased frequencies of polychromatic erythrocytes with micronuclei have been found by numerous workers following the exposure of mice to benzene by various routes. Hite et al. (1980) found such increased frequencies at doses higher than 0.125 ml/kg following the oral administration of benzene to male and female mice in 2 consecutive doses, at concentrations ranging from 0.062 to 2.0 ml/kg. It is not clear from the paper whether the increased frequencies were observed in both males and females. Toft et al. (1982) demonstrated increased frequencies of micronuclei at concentrations greater than 21 ppm subsequent to the exposure of male mice by inhalation, for periods of 4 to 10 days, to benzene concentrations of between 1 and 200 ppm.

Male mice were found by Meyne and Legator (1980) to be more sensitive to the micronucleus-inducing effects of benzene than were females, following the oral or i.p. administration of doses of benzene between 0.1 and 1 ml/kg. The lowest effective dose by both routes was 0.5 ml/kg. These results are similar to those of Siou, Conan et al. (1980) who used oral doses of up to 2.5 ml/kg. Siou, Sourdeix et al. (1980) showed that at the same dose the male sensitivity, following castration, was reduced to that of the females. The sensitivity reverted to that of normal males when testosterone was administered. A similar sex difference has been reported by Gad-el-Karim et al. (1984) following oral administration of benzene to mice after pre-treatment with toluene or 3-methylcholanthrene. This study reinforces the observation of hormonal influences as an etiological factor in the greater female resistance to benzene clastogenicity.

- b) Metaphase analysis. Styles and Richardson (1984) studied chromosomal damage in male rats given a single 6-hour exposure to benzene by inhalation, at levels of 1, 10, 100 and 1000 ppm. Although a dose response was observed over all 4 dose levels, statistically-significant effects were found only at 100 and 1000 ppm, the responses at 1 and 10 ppm being within the frequencies for the historical controls.

Tice et al.(1982) found a non-statistically-significant increase in chromosome aberrations in the bone-marrow of mice after exposure by inhalation to 3000 ppm of benzene for 4 h. Styles and Richardson (paper in preparation) found that after multiple dosing of rats with benzene by inhalation, 6h/d for 5 days, chromosomal aberrations were induced at 1000, but not at 100,10 or 1 ppm.

In a study for the American Petroleum Institute (Hazelton Lab., 1983), male and female CD-1 mice and Sprague-Dawley rats were exposed by inhalation to levels of 0, 1, 10, 30 or 300 ppm of benzene for 13 weeks. Following this exposure, chromosomal analysis was performed on the bone marrow cells. No increase in chromosomal damage was observed in either the rats or mice exposed at the 1, 10 or 30 ppm levels. Slight increases in aberrations were observed in all animals at the 300 ppm exposure level, but these proved to be statistically significant only in female mice. Therefore it was concluded that no clastogenic effects were apparent at levels of benzene of 30 ppm or lower.

The differences in quantitative response in these metaphase analysis studies may be tentatively explained by differences in the strain of animals used and in the duration of treatment. It is difficult to draw any conclusions from them with respect to the adequacy of current human exposure standards.

- c) Sister-chromatid exchange (SCE). It has been reported by Tice et al.(1981) that statistically-significant increases in SCE levels were induced in the bone marrow cells of mice given a single exposure to atmospheric levels of benzene of between 28 and 112 ppm, for 4 hours.

1.2. Postulated mechanism of clastogenicity of benzene

A critical review of the genotoxicity of benzene has been made by van Raalte and Grasso (1982) who draw attention to recent evidence suggesting that clastogenic activity may be due to the binding of benzene, or its metabolites, to tubulin and the consequent disruption of microtubules, which are essential structures for the orderly arrangement of chromosomes.

Siou and Conan (1978) and Siou et al.(1981) first reported that male mice were more sensitive than were females to the clastogenic effect of benzene. Recent papers by Tice et al.(1982) and Gad-el-Karim et al.(1984) confirm this intersexual difference and the probable role of hormonal factors. However, these observations are not entirely supported by the above API study in which both sexes gave a similar response. This apparent discrepancy may be due to the prolonged exposure period in the API study compared with those mentioned above.

The effect of modifying the mixed-function oxidase activity and liver metabolism have been studied. Neither partial hepatectomy (Tice et al.,1982) nor the inhibition of liver metabolism by SKF 525A (Gad-el-Karim et al.,1984) modified the clastogenic activity of benzene when administered acutely to mice. Enzyme induction by Aroclor 1524 and phenobarbital also failed to affect this activity, whereas induction by 3-methylcholanthrene enhanced the clastogenic activity of benzene in mice (Gad-el-Karim et al.,1984). Toluene, which competes with the metabolism of benzene, antagonised the clastogenic effects of benzene in mice treated by inhalation (Tice et al., 1982), subcutaneous injection (Tunek et al.,1982) and oral gavage (Gad-el-Karim et al.,1984).

The role of the hydroxylated metabolites of benzene has been studied by Tunek et al.(1982) who subcutaneously injected mice with benzene, hydroquinone and catechol. Only benzene and hydroquinone were clastogenic. Morimoto et al.(1983) tested benzene, phenol, hydroquinone and catechol on human lymphocyte cultures in vitro, with and without metabolic activation by rat-liver microsomes supplemented by an NADPH-generating system. To reach the maximum frequency of SCE, benzene and phenol required much higher concentrations of the metabolic activation mixture than did catechol and hydroquinone.

The above results suggest that the clastogenic potential of benzene is due, at least in part, to its hydroxylated metabolites.

2. Data on Humans

Fleig and Thiess (1980) studied a group of workers exposed to a number of chemicals, including benzene, for possible cytogenetic effects. Chromosome analyses on lymphocytes showed that there was no increase in chromosome aberrations in those workers exposed to benzene. The paper gives no indication of exposure levels, the control groups used, or the intervals at which monitoring took place.

Watanabe et al.(1980) found no evidence of any increase in the frequency of chromosomal aberrations or SCE in a group of 16 females exposed to benzene at concentrations of up to 40 ppm, for periods of up to 20 years, while painting ceramic wares.

Clare et al.(1984) measured the levels of phenol in the urine of 10 workers and confirmed that they had been exposed to high concentrations of benzene during a spillage. Blood analyses of these workers 3 months later gave no evidence that exposure to benzene had caused any lasting chromosome damage.

Sarto et al. (1984) found no statistically-significant increase in the frequency of sister chromatid exchange in the lymphocytes of workers exposed to concentrations of benzene between 0.2 and 12.4 ppm. The levels of chromosomal aberrations estimated in blood samples from the same individuals showed that there was no statistically-significant increase in the percentage of total aberrations between the exposed and a control group. However, a small but statistically-significant difference was observed when the ratio of chromatid to chromosome aberrations was considered. Since the exact type of chromosomal aberrations was not specified, it is not possible to relate these findings to aberrations reported at higher benzene concentrations, and thus to assess whether the small difference found by Sarto et al. was truly related to exposure to benzene or was more likely to have been a reflection of normal background variations. Only the range of benzene concentrations was given in the paper, and therefore the increased incidence in chromosome-type aberrations cannot be related to any particular concentration. The study covered a period of at least 11 years, and it would

have been useful to know what the trend of benzene concentration was over the years.

Funes-Cravioto et al.(1977) studied the lymphocytes from 73 workers in several chemical laboratories, and in the printing industry, where exposure to organic solvents including benzene was common to all work environments. They found a significant increase in the frequency of chromosome breaks compared with that in the cells of 49 control subjects. No individual chemical could be singled out as the causative agent.

3. Conclusions

Benzene is a clastogen in in vitro and in vivo test systems. In both systems male animals and cells are in general more sensitive to the clastogenic effects than are female animals or cells. In in vitro studies on benzene it is essential to optimise the auxiliary metabolic activation systems before a positive result can be obtained.

The lowest dose levels which have been shown to produce statistically -significant chromosomal effects in exposed animals are 100 ppm for chromosomal aberrations, 28 ppm for SCEs and 21 ppm for micronuclei. However, it is not realistic to expect the same quantitative response in man at the doses found to produce effects in animals.

The Task Force agrees with the statement in the CEFIC (1983, p.29) report, that no relationship between the types of chromosome damage observed and effects on human health can be established at present. The observations have to be taken as indicating a response to exposure to benzene of unknown biological significance. The recent papers on human cytogenetics do not help to clarify the situation.

E. EPIDEMIOLOGY

It is well known that the exposure of people to concentrations of benzene vapour greater than 30 ppm may induce adverse haematological effects characterised by a depression of haematopoiesis and a reduction in the number of circulating red blood cells, white blood cells and platelets, which may culminate in aplastic anaemia if exposure continues. These haematotoxic effects can be demonstrated in experimental animal studies (IARC, 1982) and have been confirmed in humans by numerous case reports and epidemiological studies (IARC, 1982; Laskin and Goldstein, 1977; NRC, 1976; Health and Safety Executive, 1982; CEFIC, 1983; van Raalte and Grasso, 1982; Goldstein, 1983). If exposure is discontinued, recovery from the haematotoxic effects usually occurs (Jandl, 1977).

A causal association between regular and prolonged exposure to high concentrations of benzene (greater than 100 ppm) and leukaemia in humans is also well established. The evidence for this association has been fully reviewed in recent publications (IARC, 1982; Health and Safety Executive, 1982; CEFIC, 1983; van Raalte and Grasso, 1982; Goldstein, 1983).

A number of cases of leukaemia have been reported following recovery from earlier episodes of benzene-induced blood anomalies (Girard et al., 1970; Aksoy, 1980; Goguel et al., 1967) and it has been argued that benzene-induced leukaemia develops only if some degree of blood disease caused by excessive exposure to benzene has occurred in the past (Jandl, 1977).

It is generally agreed that the most frequently encountered form of leukaemia following excessive exposure to benzene is the acute myelocytic form, although a number of reports suggest that the incidence of other varieties of leukaemia (acute and chronic monocytic or lymphatic, and chronic myeloid, leukaemia) may also be increased (Girard et al., 1970; Aksoy, 1980; Goguel et al., 1967; Girard et al., 1971). Evidence has been published that the incidence of lymphopoietic cancers, ie. Hodgkins disease and malignant lymphomas, may also increase following exposure to benzene (Vianna and Polan, 1979; Aksoy et al., 1974a; Bousser et al., 1947) although a study by Smith and Lickiss (1980) does not confirm this association. The possibility that multiple myeloma may be linked with benzene exposure has been proposed by Decoufle et al. (1983). Overall, these reports are too few in number, and the evidence quoted in

their support is insufficient, to make a convincing case that cancers other than acute myelocytic leukaemia can be causally associated with excessive exposure to benzene.

1. Recent Findings

Since the end of 1982 a number of additional epidemiological studies have been published.

1.1. The Chemical Manufacturers' Association in the USA sponsored an investigation by Wong et al.(1983) on the mortality of chemical workers occupationally exposed to benzene. A comprehensive historical/prospective mortality study of 4602 male chemical workers exposed to benzene for at least 6 months, between 1 Jan. 1946 and 31 Dec. 1975, was carried out. 3074 workers employed in the same plants for 6 months or more during the same period of time, and with no occupational exposure to benzene, were used as a control group. The 4602 exposed workers were divided into two groups : (i) continuously exposed (potential exposure to benzene for at least 3 days in each week), and (ii) intermittently exposed (casual or periodical employment in areas of potential benzene exposure). This division was made on the basis of historical job evaluations and a retrospective estimation of exposures to benzene likely to have been experienced by the workers during their employment. For the continuously-exposed group, exposures were calculated according to job allocation in terms of "maximum peak" exposures experienced in the course of their entire exposure history, and of daily 8-hour time-weighted-averages expressed as cumulative ppm-months.

Jobs involving intermittent exposure were arbitrarily divided into the following exposure groups :

	<u>8hr TWA</u>	<u>Peaks</u>
Low	< 1 ppm	< 25 ppm
Medium	1-10 ppm	25-100 ppm
High	11-50 ppm	> 100 ppm
Very high	> 50 ppm	

For the intermittently exposed workers only estimated peak exposures were used. No data are included on the numbers of workers allocated to each of these exposure groups. The inaccuracies inherent in this method of calculating benzene exposures make it very difficult to derive any realistic

dose-response relationships. The mortality in the 4,602 continuously and intermittently exposed workers and in the 3,074 unexposed population were compared against each other and with the 1946-77 National age/cause/-race-specific mortality for males, with Standardised Mortality Ratios (SMRs) as the basis of comparison.

The results showed :

- similar or lower SMRs for all causes of death of the exposed population (continuous and intermittent) compared to the national reference population. There was a number of slightly-raised SMRs but none was statistically significant, usually because the number of deaths in each category was too low for statistical evaluation.
- a number of significantly higher relative risks (RRs) when the combined exposed population (continuous and intermittent) was compared with the unexposed population of workers. Overall there was an RR of borderline statistical significance for lymphopoietic cancer in the combined exposed group. For the continuously-exposed workers there was a significantly higher RR for lymphopoietic cancer.
- a statistically-significant dose-response relationship between cumulative exposure to benzene (ppm-months) and i) mortality from all lymphopoietic cancers combined, and ii) leukaemia.

A relative risk could not be computed for leukaemia as there were no cases observed in the unexposed population, but when the Mantsel-Haenszel chi-square statistical procedure was applied there was a significant mortality from leukaemia (of all sorts) in the continuously-exposed population compared with the unexposed population.

There were some unusual findings in the study :

- of the 7 recorded leukaemia deaths in the exposed population, none was of the acute myelocytic variety which is most commonly associated with benzene exposure.
- although 3.40 deaths from leukaemia could have been expected, no leukaemia deaths were reported in the control group of unexposed workers. On the basis of a Poisson distribution, the likelihood of this event occurring by chance is 3 in 100, a statistically-significant discrepancy which suggests that the two populations were not comparable in some other important but undefined quality.

- In a critique of this paper, Rothman (1984) makes the following points :
- the overall death rate reported in the unexposed control population is far below the expected figures based on the general population rates, and the discrepancy is in excess of the usual healthy-worker effect.
 - the control population also has a smaller relative risk than the exposed group for 17 out of 21 independent broad categories of death.
 - while it is possible that benzene may be causally-related to one or several categories of death, the great discrepancy in mortality patterns is not plausibly explained by chance or by a biological effect of exposure. The most likely explanation is that there is some unidentified difference between the two populations which makes them unsuitable for comparison.

In this letter Rothman also makes a number of serious criticisms of the methodology of the study and concludes that in the light of its shortcomings it cannot be evaluated. In discussing their results, Wong et al. (1983) give a very fair account of several limitations in the study which add to the doubts on the validity of their conclusions. One of the greatest weaknesses is the failure to quantify accurately the exposure to benzene experienced by the exposed group of workers. The figures given in the report are no better than "best guesstimates" and make no useful contribution towards determining the shape of the dose-response curve relating leukaemia incidence and exposure to benzene, at its lower end.

In summary, this paper adds little to our knowledge of the carcinogenic or toxic properties of benzene, except, perhaps, to introduce further questions on whether there is a causal relationship between exposure to benzene and a much wider range of leukaemias and lymphopietic cancers than hitherto acknowledged. The number of deaths attributable to these causes is too small to allow any valid conclusion to be reached. Without more accurate data on exposure to benzene, no meaningful assessment of the increased risk of developing leukaemia reported in this publication is possible.

- 1.2. Decouflé et al. (1983) reported a historical mortality study on 259 male workers employed for any length of time between 1/1/47 and 31/12/60 at a chemical plant where large quantities of benzene had been used during the period 1950-1961. Unfortunately, no quantitative measurements of benzene

exposure were available from the plant and the authors merely state that "... we can safely say that benzene exposures resulted largely from multiple fugitive emissions peculiar to past process technology as well as the particular quality control and maintenance procedures practised in the past." Anecdotal reports from plant operatives suggested that some workers "received benzene exposures that were in excess of levels given off by the 'ordinary run' of the manufacturing process". The authors also point out that chemicals other than benzene were present and accounted for a proportion of the total exposure experienced by the workforce. Four deaths from lympho-reticular cancers were observed where 1.1 would have been expected by comparison with the 1947-1975 national mortality rates of the U.S. white male population - a statistically significant difference at the 0.05 level. From death certificates, inquiries made of next of kin, and medical records of the deceased, the four deaths from lympho-reticular cancers were broken down into the following categories :

Leukaemia

Acute monocytic	1
Chronic lymphatic	1
Acute myelomonocytic (developing	1
2 years after start of radio- and	
chemo-therapy for multiple myeloma)	
Multiple myeloma	1

The authors conclude that their findings are consistent with previous reports of an increased incidence of leukaemia following occupational exposure to benzene and suggest that there may be an association between benzene exposure and the development of multiple myeloma although the latter association is not supported by the findings of Wong et al.(1983). However, the absence of any objective benzene-in-air measurements, the unusual variety and small number of leukaemias observed, the possible concomitant exposure of the workforce to other chemicals and the small size of the study population make it difficult to place any reliance on their conclusions. The study makes no contribution to our understanding of the dose-response relationship between benzene exposure and the development of benzene-induced leukaemia.

- 1.3. Tsai et al.(1983) examined the mortality experience of 454 male workers employed in a large Texas oil refinery for any length of time between Sept. 1952 and Jan. 1978. Of the cohort, 359 had been employed during this period for at least 1 year. Benzene exposure measurements taken by personal sampling were available only for the years 1973 through 1982; 96% of the samples contained less than 5 ppm, and 84% less than 1 ppm. The authors assumed that benzene exposures prior to 1973 would have been of the same order, although it seems more likely that exposures dating as far back as 1952 were higher than those recorded since 1973. No cases of lymphopoietic cancer (including leukaemia and aleukaemia) were recorded for the total cohort, against an expected number of 1.12 deaths based on U.S. white and non-white mortality rates (not a statistically significant difference).

This finding is in agreement with the results of a comparable mortality study conducted on 35,000 workers employed over a 25-year period in 8 oil refineries in the UK (Rushton and Alderson, 1981), but is clearly at variance with a study by Rinsky et al.(1981) in which 7 deaths from leukaemia were reported in a population of 748 workers exposed to benzene whilst engaged in the manufacture of rubber hydrochloride between 1940 and the end of 1949. However, the true concentrations of benzene in air to which the Rinsky et al. and Tsai et al. populations were actually exposed is a matter of considerable doubt. Until it can be established more precisely what the benzene exposures of the two study populations were, any comparison of their mortality rates is invalid.

- 1.4. Workers in rubber industry. In addition to the Rinsky work mentioned above, a number of mortality studies on workers in the rubber industry have shown an increase in deaths from cancer of the haematopoietic and lymphatic systems (McMichael et al.,1974, 1975, 1976; Anjelkovic et al.,1976; Manson and Makano, 1971; Delzell and Manson, 1981; Infante and White, 1983), although a study by Parkes et al.(1982) throws doubt on the association with lymphatic leukaemia.

Attempts to identify causative factors have focussed on exposure to benzene, but the evidence from these studies incriminating benzene, as opposed to the other chemicals and solvents present in the workplace, is unconvincing. A case-control study based on cases of lymphatic leukaemia

previously reported by McMichael et al.(1975), and later studied by Wolf et al.(1981), presents evidence that there is a stronger association between lymphatic leukaemia and exposure to a number of other solvents used in the rubber industry than between lymphatic leukaemia and exposure to benzene (Checkoway et al.,1984).

In Finland, Kilpikari (1982) investigated the mortality experience of 784 male workers employed in a plant processing rubber for footwear and tyre manufacture and compared their findings with the specific mortality rates for males in the whole of Finland. Overall, the observed and expected number of deaths were equal although there was a slightly increased relative risk of death from cancer (Obs/Exp=5/2.9, $p=0.05$) when employment in the processes exceeded 10 years. No workplace exposure measurements were quoted, nor was any breakdown given of the solvents or other chemicals used in the two processing departments.

- 1.5. Chinese study. Yin Songnian et al.(1983) reported the results of a study of 578,729 workers in China drawn from 27,808 factories in which benzene was used as a solvent or chemical intermediate. Benzene exposures in 19,969 factories ranged from 0.66-844 mg/m³ (0.22-281 ppm). Benzene in air concentrations were above 1000 mg/m³ in 1.3% of the measurements. The authors report that 2,676 cases of benzene poisoning were observed, including 24 cases of aplastic anaemia and 9 cases of leukaemia. No account is given of the length of time the workforce was exposed to benzene.

The failure of the authors to define sufficiently clearly their methods and their criteria for diagnosing "benzene poisoning" make it difficult to evaluate their findings. They make no attempt to describe how data obtained from so many different sources have been standardised in order to make a homogeneous population of exposed workers on which reliable conclusions can be based.

- 1.6. Reproductive effects. Davis (1983) has reviewed the reproductive effects of benzene and quotes a number of epidemiological studies suggesting that occupational exposures of either parent to hydrocarbon solvents may be associated with an increased risk of cancer in their children. Such an association was not found by Zack et al.(1980). A publication by Hemminki

et al.(1983) indicated that women employed in the rubber industry in Finland may be at risk of increased spontaneous abortions. Other studies in Finland (Olsen, 1983; Holmberg, 1979) suggest an association between parental exposure to organic solvents and the birth of children with congenital malformations of the central nervous system.

Pinpointing the causative chemicals in the above studies remains impossible. Benzene could be a candidate, but since its use as a solvent has been discontinued for many years and it would probably have been present only as a minor impurity in other solvents, it seems unlikely that it was the causative agent. The many limitations of epidemiological studies on human reproductive effects, including difficulties in the identification of exposed populations, reliability of exposure data, lack of knowledge of the background incidence, bias by confounding factors and the anecdotal nature of the information analysed, make it impossible to reach any conclusions about the effect of benzene from the evidence presented in these reports.

- 1.7. Exposure of the general population . Measurements of benzene concentrations in rural community air are reported (IARC, 1982, p.99) to range from 0.3 to 54 $\mu\text{g}/\text{m}^3$ (0.1 - 17 ppb). The same authors (p.101) give values for benzene in air at urban locations, most of which lie in the range 5 to about 200 $\mu\text{g}/\text{m}^3$ (1.6-66 ppb) with a few in the range of about 200 to 573 $\mu\text{g}/\text{m}^3$ (66-191 ppb). In a survey of 22 states in the USA (IARC, 1982) it was estimated that about 6 million people were exposed to 0.3-3 $\mu\text{g}/\text{m}^3$, about 1 million to 3-13 $\mu\text{g}/\text{m}^3$, about 200,000 to 13-32 $\mu\text{g}/\text{m}^3$, and about 80,000 to more than 32 $\mu\text{g}/\text{m}^3$ (32 mg/m^3 = 10ppb) of benzene.

Using data from studies by Rinsky et al. (1981), Aksoy et al., (1974b) and Ott (1978), the EPA (1984, page 23488) has estimated the additional risk of dying from leukaemia by exposure to benzene. Their estimate is that a lifetime exposure to 1 ppm (3.2 $\mu\text{g}/\text{m}^3$) would result in 22 additional deaths in 1000 people, corresponding to a relative risk of 5.

In Appendix 1 the Task Force has made the following calculation relating to the EPA estimates. A lifetime exposure to 10 ppb (32 $\mu\text{g}/\text{m}^3$) would result in a relative risk of 1.04. To detect a relative risk of 1.04 at a significance level of 5% and a power of 90%, an epidemiological study on a

population of 0.9 million, over their lifetime of 75 years, would be required. Since a study of this size and duration cannot, in practice, be carried out, such hypothetical risk estimates could never be confirmed. Furthermore, in a number of publications serious criticism has been made of the validity of the exposure data on which the EPA based their calculations (van Raalte and Grasso, 1982 ; Health and Safety Executive, 1982 ; CEFIC, 1983). Whatever the merits or defects of the mathematical model used, it follows that if the basic data are open to question so must be the reliability of the risk estimate.

The greatest single contribution to the presence of benzene in community air comes from the use of motor gasoline. Allowing a latent period of up to 15 years between the onset of exposure to benzene and the development of leukaemia, Van Raalte (1982) has compared the post-war increase in the consumption of gasoline with national mortality rates from leukaemia in three European countries. He concludes that the incidence of leukaemia has not risen to reflect the increasing emission of benzene from gasoline into community air and that, at low concentrations, benzene has little or no effect on this incidence.

Funcke and Hamacker (1982) studied an urban population exposed to a long-term average benzene concentration of 0.05 mg/m^3 (about 0.02 ppm) or lower, near a coking plant in Germany. They observed a relative increase in the number of circulating lymphocytes in half the population under investigation and ascribed the effect to benzene exposure. The authors, however, quote in their paper the following evaluation by Prof. van de Loo of the University of Münster, here translated from the German : The observed changes perhaps represent a biologically adverse effect, but on the basis of the methodology used are of limited interpretability. A causal relationship between benzene burden and blood abnormalities cannot, in this case, be regarded as validated.

The Task Force notes, furthermore, that a persistent relative lymphocytosis in the absence of any other haematological abnormality has not previously been reported as a toxic manifestation of the exposure of humans to benzene, even at high concentrations.

2. Conclusions

Voluminous case history reports and epidemiological studies, dating as far back as 1897, demonstrate beyond reasonable doubt that exposure of people to benzene can be associated with the onset of haematotoxic (at above 30 ppm) and leukaemogenic (at above 100 ppm) effects. There is general agreement that the most common carcinogenic manifestation of exposure to benzene is acute myelocytic leukaemia. Whether the incidence of other forms of lymphopoietic or haematopoietic cancers is also increased remains unresolved, and is likely to remain so under the conditions of modern benzene-using operations in which the number of persons exposed is small and the degree of exposure is low. More importantly, all the literature published to date on the consequences to health of exposure to benzene lacks, without exception, accurate or sufficient data on the quantitative exposure to benzene of the persons or populations under study. Without reliable and quantified exposure data it is impossible to describe, with any degree of conviction, the shape of the dose-response curve relating exposure with any of the recorded toxic effects. From such data as is available, it is impossible to define a "no-effect level" for exposure to benzene upon which regulating authorities may base standards or limits governing permissible levels of exposure. It is because precise evidence of the above kind is missing that much controversy still continues on what level of exposure to benzene is acceptable to all sections of the community. On balance, the information reviewed in this section does not provide convincing evidence that persons exposed to benzene at the commonly-adopted exposure standard of 10 ppm suffer from an increased incidence of leukaemia or other forms of cancer. If this estimate is to be verified, future epidemiological studies must at least satisfy the following requirements :

- they should give accurate and systematic measurements of benzene-in-air concentrations experienced by the population under study throughout the whole period of their exposure to benzene.
- populations under study must be large enough to detect, with confidence, small increases in the incidence of cancer morbidity and mortality.
- they should take full account of exposure to other chemicals and of influences which may distort the results of the study.

Until these requirements can be met there is nothing to be gained in continuing to report the mortality or morbidity of populations exposed to benzene under inadequately-defined circumstances.

F. CONCLUSIONS

1. Recent animal studies show that benzene, in addition to leukaemia, can induce other types of malignant changes in animals. There is no evidence to confirm that malignancies other than leukaemia occur in man.
2. The recent studies reviewed in this report provide no convincing medical or toxicological evidence for amending current benzene exposure standards.

G. RECOMMENDATIONS

1. Further studies are needed to elucidate the mechanism, and significance for human health, of clastogenic effects in general, and of those associated with exposure to low levels of benzene in particular. No other long-term animal studies are justified until this work has been carried out.
2. Comprehensive health records of selected populations exposed to measured levels of benzene should be maintained. Data on cancers at sites other than the blood-forming organs should be included.
3. Regular and systematic measurements of exposure to benzene, on which to base future epidemiological studies, should be made.
4. Future epidemiological studies should satisfy the following requirements:
 - they should be based on accurate and systematic measurements of benzene-in-air concentrations experienced by the population under study throughout the whole period of their exposure to benzene.
 - populations under study must be large enough to detect, with confidence, small increases in the incidence of cancer morbidity and mortality.
 - they should take full account of exposure to other chemicals and of influences which may distort the results of the study.

H. APPENDICES

Appendix 1. Number of Persons Required in a Cohort Study to Give Reliable Estimates of Relative Risk

The incidence of leukaemia cases in any segment of the general population is assumed to follow a Poisson distribution, with an average incidence rate E . If the incidence is greater in some sub-group of the population, it is assumed that the increased incidence will also follow a Poisson distribution with an average RE , where R is the relative risk.

The probability, α , that an observed increased incidence ($>E$) has arisen in a sub-group that has the normal incidence rate E can be calculated. If α is less than 0.05 (5%), the increased incidence is usually described as statistically significant, and the interpretation is that the incidence rate in the sub-group is not really E . In addition, the probability, β , that the observed incidence ($>E$) can be attributed to the sub-group having an increased incidence rate RE can be estimated. The quantity $1-\beta$ is called the power of the study.

To be adequate for decision making, an epidemiological study should be able to detect an increased incidence rate in the exposed group at the 5% significance level (α), and it should have a power of 80-90% for a suitably-chosen relative risk R . This means that the incidence which would have a 5% chance of occurring when the average incidence is E is also the incidence that would have 10-20% chance of occurring when the average incidence is RE . In simple language, there should be a sufficient difference between the Poisson distributions with average E and RE for the overlap to be small. If the average of a Poisson distribution is more than 10, the distribution is considered to be normal, with a standard deviation equal to the square root of the average.

These requirements for (α) and ($1-\beta$) can be expressed mathematically :

$$E + Z_{\alpha} \sqrt{E} = RE - Z_{\beta} \sqrt{RE} \quad (1)$$

$$RE = \left(\frac{Z_{\alpha} + Z_{\beta} \sqrt{R}}{R-1} \right)^2 \times R \quad (2)$$

where Z_{α} = the standard normal deviation at probability α

Z_{β} = the standard normal deviation at probability β

RE = required number of leukaemia deaths in a study population with

increased risk.

In Table 1 are given the values of Z_α and Z_β corresponding to various values of α and β :

Table 1

<u>Values of α or β , %</u>	<u>Z_α, Z_β</u>
1	2.3268
5	1.6452
10	1.2817
20	0.8415

In Table 2 the required number of leukaemia deaths and the corresponding required sample size of the study population (follow-up period, 75 yr) for various relative risks are given. These figures are based on equation (2), with:

- average leukaemia mortality in general population, 8 in 10^5 per year (derived from Levin's figure in footnote);
- significance level, α , 5%;
- power 90%, ie. $\beta = 0.10$;
- $Z_\alpha = 1.6452$ and $Z_\beta = 1.2817$ (see Table 1).

Table 2

<u>Relative risk</u>	<u>Required leukaemia deaths in study population</u>	<u>Required sample size of study population (75 yr follow-up)</u>
2	24	1993
1.5	63	6891
1.1	984	1.49×10^5
1.04	5666	9.08×10^5
1.01	86,903	1.43×10^7
1.005	3.45×10^5	5.72×10^7
1.001	8.58×10^6	1.43×10^9

Footnote : Levin et al. (1974) have estimated that in the white, male population of the USA the leukaemia/aleukaemia death rate is 6 in 1000 people over their lifetime, assuming a lifetime of 75 years.

Appendix 2. Glossary of Terms

Leukaemia	- A malignant disease of the blood-forming organs characterised by an increase in the white or, much more rarely, red cells in the peripheral blood, and by the appearance of abnormal cells. It is called chronic when it lasts for several years and acute when death ensues within months or a few years of diagnosis.
Myelocytic (or myelogenous leukaemia)	- A leukaemia affecting the phagocytic white cell (myelocyte) series of the blood.
Lymphocytic (or lymphatic) leukaemia	- A leukaemia affecting the lymphocytes.
Lymphoma	- A malignant disease of lymph nodes. If the lymphocytes in the blood are excessively increased the disease is also called lymphocytic leukaemia.
Leukopenia	- Reduction of the number of white blood cells in the peripheral blood.
Haematopoietic hyperplasia	- Increased cellularity of the red and white blood-forming cells in the bone marrow.
Lymphocytopenia	- A reduction of lymphocytes in the peripheral blood.
Myeloma	- A malignant disease of the plasma cells (B-lymphocytes). If it affects more than one organ it is called multiple myeloma.
Lympho-reticular	- A collective term employed to denote tumours of the lymphatic and sometimes (incorrectly) of the myelocytic systems.

Aleukaemia

- A malignant disease of the blood-forming tissues which is confined to the bone-marrow. No changes are detectable in the blood.

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