CHAPTER 1

CARCINOGENICITY OF BESZENE, TOLCENE AND XYLENE: EPIDEMIOLOGICAL AND EXPERIMENTAL EVIDESCE

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INTRODUCTION

Benzene was first isolated in 1825, and commercially recovered from coal-tar-derived light oil in 1849 and from petroleum in 1941 (IARC, 1982). By the late 1950's, most commercial benzene was petroleum-derived.

Chronic human exposure[1] to benzene may result in adverse haematological effects, characterized by a variety of blood dyscrasias, including leucopenia, thrombocytopenia, pancytopenia and anaemia. Benzene is also considered to be a human carcinogen and is well established as a cause of leukaemia (IARC, 1982). It is recognized as being carcinogenic by the governments of Finland, the Federal Republic of Germany, Italy, Japan, Sweden, Switzerland and the U.S.A.

By comparison with benzene, there is no direct epidemiological evidence concerning the carcinogenicity of toluene and xylenes, and only limited experimental evidence from animal studies.

EVIDENCE OF BENZENE CARCINOGENICITY IN HUMANS

Leukaemia and other lympho-haematopoietic cancers: early studies

There have been many case reports, case series and epidemiological studies associating exposure to benzene with leukaemia in humans. However, very few of the studies have provided quantitative information about the relative risk of leukaemia under specified conditions of exposure.

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[1] Exposure concentrations are given throughout in parts per million - ppm. To convert to ISO recommended units, for benzene, 1 ppm = 3.25 mg m^{-3} (~ 20 C).

The first case report suggesting an association between exposure to benzene and leukaemia was given by Delore and Borgomano (1928). An acute lymphoblastic leukaemia was observed in a worker in France. who was exposed to benzene for five years. Since then. numerous reports describing leukaemia cases among persons exposed to benzene nave appeared. predominantly from Italy. France and Turkey (Goldstein. 1983).

Several pioneering epidemiological studies investigating the relationship between exposure to benzene and leukaemia. as well as other lympho-haematopoietic cancers. were published in the mid-1970's. **Aksoy** published a number of reports on the risk of leukaemia among Istanbul shoe makers exposed to benzene-containing solvents (Aksoy *et al.*, 1972, 1974; Aksoy, 1980). He estimated that the annual crude incidence rate *of* leukaemia among the shoe makers was 13.5 per 100000. compared with an overall annual incidence rate in the general population of 6 per 100 000.

Vigliani (1976). reviewing earlier studies (Vigliani & Saita, 1964), estimated the increase in risk *of* leukaemia to be 70-fold for workers exposed to benzene in shoe factories in Italy. As with Aksoy's studies. the apparent levels of exposure to benzene were predominantly within the 200–500 ppm range. However. these exposure data uere too imprecise for further quantitative analysis.

Thorpe (1974) reported an epidemiological study of leukaemia incidence among workers from eight Esso Perroleum affiliates in Europe. Employees were divided into two groups: those with a potential exposure Io 1% or more benzene over a five-year period and those not exposed or only occasionally exposed. Among the exposed. 8 leukaemia deaths were observed. compared to the expected 6.6. yielding an age-standardized mortality ratio (SMR) of 121 (not statistically significant). Among the non-exposed. the number of deaths observed was 10 and the expected figure was 16.7. the corresponding SMR being 60 (not statistically significant). These figures indicate that the employees exposed to benzene experienced an approximately two-fold risk of leukaemia when compared to non-exposed employees. No benzene exposure levels were available from the study.

Epidemiological studies enabling quantitative estimates of risk for leukaemia

The results of a U.S. National Institute of Occupational Safety and Health (NIOSH) stud! of 748 white male workers exposed to benzene during the manufacture of rubber hydrochloride have been published on three occasions (Infante *et al.*, 1977, 1979; Rinsky *et al.*, 1981). The first two papers uere based on the results with 75% complete follow-up of vital status. uhile the Rinsky *et al.* (1981) paper was based on data with 98% complete follow-up. The findings were essentially the same in each *of* the three papers: there was a three-fold increase in the risk of death from lympho-haematopoietic cancers and an almost six-fold increase for leukaemia specifically (7 leukaemia deaths *versus* 1.25 expected). Among workers employed for more than 5 years, 5 had died from leukaemia. compared to 0.23 expected. indicating a 21-fold increase in risk

Rinsky *et al.* (1981) devoted considerable discussion Io the industrial hygiene measurements available for one of the two study locations in Ohio. However. the investigators made no link between the available benzene measurements and the

Table 1 Estimate of excess risk of leukaemia deaths per 1 000 workers exposed to benzene'

Number of years exposed	Exposure level			
	10 ppm		1 ppm	
	NIOSH study[a]	Dow study[c]	NIOSH study	Dow study
45[d]	44–152	48–136	5–16	5–15
30	30–104	32–93	3–11	2–10
15	15–54	16–48	15–5	2–5
5	5–18	5–16	05–2	0 5–2
1	1–4	1–3	0 1–0.4	0 1–0.3

[a] From while *et al* (1982)
[b] Rinsky er *al* 1981)
[c] On *et al* (1978)
[d] 45 years = occupational lifetime

employment histories of the individual cohort members. (Subsequently, Crump and Allen (1984) and Rinsky *et al.* (1985) did link these data in unpublished risk assessments. Cumulative benzene exposures of individual uorkers in this cohort were estimated from job-exposure matrices. This approach indicated a heightened. 45-fold. increase in risk of leukaemia in the highest cumulative exposure category (Rinsky *et al.*, 1985). See also Appendix, p. 18.)

Ott *et al.* (1978) reported the mortality of a cohort of 594 Dow Chemical workers exposed to benzene in the production of chlorobenzol, alkyl benzene and ethyl cellulose from 1938 to 1970. Their mortality experience was observed from 1940 through 1973. With expected deaths based on U.S. white male mortality, no statistically significant increases were found in any cause of death for the cohort as a whole. even when mortality was analysed by estimated cumulative benzene dose (ppm-months) and latency However. further review of the three cases of leukaemia showed all three to be myelocytic. with two being classified as acute. Based on incidence data reported in the U.S. Third National Cancer Survey, the expected incidence of myelocytic leukaemia was only 0.8 cases.

Because the Occupational Safety and Health Administration in the U.S.A. is legally required to consider quantitative risk assessments whenever possible from available data. such an assessment has been attempted (White *et al.*, 1982) using the above-mentioned two cohort studies. Uncertainties regarding the levels and lengths of exposure to benzene were incorporated into the analysis and the results are shown in Table 1 Based on the one-hit model, and subject to the typical uncertainties of epidemiological data. the assessment indicates that a working lifetime exposure to benzene at an exposure level of 10 ppm poses a substantial excess risk of death from leukaemia

White *et al* (1982) selected the one-hit. or linear, model because the biological mechanism of benzene-induced leukaemia is not understood well enough to justify a more complicated model. and because information from epidemiological studies regarding the relative risks of leukaemia at different levels of exposure to benzene was

Table 2. Analyses of relative risk and excess risk of death from leukaemia per 1000 workers exposed to benzene lor an occupational lifetime[a]

Authors	Relative risk	Estimated conort exposure	Excess risk per 1 000 workers	Excess risk per 1 000 exposed to	
				10 ppm	1 ppm
Ott *et al* (1978)	3.75	1–30 ppm for 8–9 years	72	24–720	2672
Infante *et al* (19771	5 6	10–100 ppm. 8 5 years average	170	17–170	17–17
Rinsky *et al* (1981)	21	10–100 ppm. ⩾ 5 years exposure	140	14–140	14–14
Vigliani (1976)	20	200–500 ppm 9 years average	475	9.5–23.8	10–24
Aksoy (1977)	25	150–210 pprn 9 7 years average	534	254-356	2.5–3.6

[a] Table adapted from Infante ana White (1985) Calculations in the last three columns were performed using the method applied by IARC 1982) to data from Infante *et al* (1977) ana Rinsky *et al* (1981)

inadequate for testing more complex models. Furthermore. as Infante and White (19851 have subsequently noted. several indications of benzene toxicity to bone marrow and peripheral blood cells have demonstrated a linear dose-response relationship.

This linear. non-threshold model assumes that ever? increment of dose is accompanied by a commensurate increment in the excess cancer **risk.** The use of this toxicologically plausible model allows extrapolation ot risks from relatively high dose levels. where cancer responses can be measured. to relatively **low** dose levels. where such risks are too small to be measured directly through animal or epidemiological studies (WHO. 1981).

The **IARC** (1982) also conducted a quantitative cancer risk assessment for benzene 3nd leukaemia using the results of four studies (Vigliani. 1976; Aksoy. 1977; Ott *et al*.. 1978; Rinsky *et al*.. 1981) IARC assumed a linear dose-response relationship. with cumulative benzene dose predicting the relative **risk** of developing leukaemia. It also made slightly different assumptions from those of White *et al.* (1982) regarding the length of exposure for the NIOSH (Rinsky) and Dow (Ott) studies. For purposes of comparison with the estimations of White *et al.* (1982). a summary of the IARC (1982)assessment of benzene risk. uith extrapolation to levels of 10 ppm and 1 ppm is shown in Table 2. The calculations in the last three columns uere performed by Infante and White (1985). using the method applied by the IARC (1982) to the NIOSH stud! and taking account of the IARC's opinion that "risk calculations (should) reflect the degree of uncertainty in the estimates of dose rate . . . by citing upper and lower bounds of such estimates" **(IARC.** 1982). The estimated excess risks of leukaemia associated with a working lifetime (45 years) ofesposure to 10 pprn and 1 ppm of benzene are similar in Tables 1 and 2.

Recognizing the need for further quantitative investigations into the relationship between benzene exposure and lymphatic and haematopoietic cancers. Wong (1983)

carried out a large industry-wide mortality study of U.S. chemical workers occupationally exposed to benzene. The population in this historical cohort study consisted of 4 602 male chemical workers from seven plants. who were occupationally exposed to benzene for at least 6 months between 1946 and 1975. and a comparison group of 3 074 male chemical workers from the same plants who were employed for at least 6 months during the same period of time. but who were never occupationally exposed to benzene. Exposure to benzene was divided into two categories: continuous exposure and intermittent exposure. Continuously-exposed workers were classified according to both an eight-hour rime-ueighted average (TWA) and a **peak** exposure level. whereas intermittently-exposed workers were characterized by a peak exposure level only. The eight-hour TWA's Here estimated by company industrial hygienists, using uniform criteria: nevertheless. it was not possible to quantify accurately the exposure of workers to benzene.

The major findings of this study were as follows. When compared to the U.S. population. the SMR's in the exposed group for all lymphatic and haematopoietic cancer combined. for leukaemia. and for non-Hodgkin's lymphoma (lymphosarcoma. reticulosarcoma and other lymphoma) were slightly. but nor significantly, higher than the national norm. When the group with no occupational exposure was used for comparison. the exposed group (continuous and intermittent) experienced a lympho-haematopoietic cancer relative risk (RR) of 2.99 (borderline statistical significance). This excess uas primarily due Io seven leukaemia deaths in the exposed group. *versus* none in the comparison group.

For the continuously exposed group. the RR for lympho-haematopoietic cancer was 3.20 ($p < 0.05$). The association between exposure IO benzene. and. specifically. leukaemia was of borderline significance **H** hen those exposed continuously and those exposed intermittently were grouped togerher. **but** the association berueen continuous exposure alone and leukaemia was statistically significant ($p < 0.05$). None of the seven leukaemia deaths was of the acute myelogenous cell type. The RR for non-Hodgkin's lymphoma among white males in the continuous]? **exposed** group was 5.02. but was not significant ($p = 0.12$).

Furthermore. the data demonstrated statistically significant dose-response relationships between the estimated cumulative exposure to benzene and mortality from all lympho-haematopoietic cancers combined and from leukaemia. Those chemical workers with a cumulative exposure of at least 720 ppm-months experienced an RR of 3.93 for lymphatic and haematopoietic cancer. when compared to the workers with no occupational exposure. However. as Wong (1983) acknowledges. the limitations in exposure estimates preclude useful dose-response analysis at low levels of exposure.

In a more recent attempt to derive quantitative **risk** assessments of leukaemia in subjects exposed to benzene. Crump and Allen (1984) used the data from Ott *et al.* (1978). Rinsky *et al.* (1981) and Wong (1983). along with animal experimental data from inhalation studies. The estimates of leukaemia risk derived by Crump and Allen (1984) from animal inhalation studies (Table 3). uhile quite consistent. are all considerably less than those made from human data (Table 2). Accepting the assumptions underlying these estimates. this indicates that rodents may be less susceptible than humans to benzene-induced leukaemia.

Table 3. Estimates' of lifetime human risk per 1 000 exposed persons' from animal inhalation studies'

Data set	Benzene air concentration (ppm)	Risk
All squamous-cell carcinomas male rats. NTP (1984)	1	0 77
	10	7 65
All squamous-cell carcinomas male mice NTP (1984)	1	2 01
	10	19 90
Leukaemia both sexes rats Maltoni *et al* (1983)	1	0 15
	10	1 46
Leukaemia male mice Goldstein *er al* (1982)	1	0 04
	10	0 38

*Assuming risk to animals is the same as risk io humans when dose is measured in mg/kg body weight/day
*Length of human exposure to benzene = 40 years
*Table based on Crumo ana Allen (1984)

Crump and Allen (1984) consider that their estimates from human studies. based upon cumulative or weighted exposure. are the most reliable. The attendant estimates of additional leukaemia cases per 1 000 workers exposed to 10 ppm of benzene range from 0.5 to 2.6 cases from one year of exposure beginning at age 20. to 15 to 88 cases from 40 years of exposure. The "window exposure" variable (defined as a shorter-term exposure occurring at the putative. latency-related. cancer induction time) tends to give lower estimates than either weighted or cumulative exposure. However. Crump and Allen (1984) judge that the estimates based on either cumulative exposure or weighted exposure are preferable to those based on window exposure.

Non-linear models would predict about the same results as linear models at high doses (10 ppm for 40 years). A person exposed to 10 ppm for 40 Fears would attain a cumulative exposure of 400 ppm-years. uhich. in the Rinsky *et al.* (1981) cohort. would place him in a category for uhich a significant excess of leukaemia occurred. Indeed. the subsequent re-analysis by Rinsky *et al.* (1985) gave an estimated 45-fold increase in risk of leukaemia for that category of exposure. Thus. no extrapolation to low doses is required to estimate risk from lifetime occupational exposure to 10 ppm.

However. the assumption of linearity plays a more imporrant role at lower exposure levels. and the estimates of risk are more uncertain. This is imporrant. since. under the benzene exposure limit of 10 ppm prevailing in the U.S.A.. the risks estimated from continuous exposure *to* 10 ppm represent a theoretical maximum. as it is unlikely that any current worker's exposure will approximate this if the 10 ppm standard is strictly enforced.

Crump and Allen (1984) point out that their estimates of risk from human data were limited to leukaemia. as this appeared to be the only type of cancer conclusively linked to exposure to benzene in humans. This approach could underestimate cancer

risk if. in fact. benzene causes other cancers. as it does in rodents. The recent epidemiology literature (Decouflé *et al.*, 1983; Rinsky *et al.*, 1985) suggests that benzene exposure may also cause multiple myeloma in humans.

Other less quantitative or less specific studies

In a cohort study of 35000 workers at 8 petroleum refineries in the U.K., the leukaemia SMR was found to be 94 (Rushton & Alderson, 1981a). Using data from this cohort study. 36 cases with leukaemia mentioned on the death certificate were the subject of a subsequent case-control study (Rushton & Alderson, 1981b). Benzene measurements were not available. but each worker was assigned. based on work histories. to either low. medium or high levels of exposure to benzene. A two-fold increase in risk was detected for workers with medium or high exposure. relative to those uith low exposure to benzene, when length of service was taken into account. This two-fold increase was of borderline statistical significance ($p = 0.05$). There was no evidence that the risk increased with length of service.

Decoufe *et al.* (1983) conducted a historical cohort mortality study of 259 male employees of a chemical plant (converted from a petroleum refinery) where benzene was used in large quantities. Unfortunately. no quantitative measures of exposure were available from the plant. Four deaths from lympho-haematopoietic cancers were observed. when only 1.06 deaths were expected: the SMR of 377 was statistically significant. Three of these four deaths uere from leukaemia: one was chronic lymphocytic. one acute monocytic and one acute myelomonocytic. This last leukaemia case also had a history of multiple myeloma. Since the fourth lympho-haematopoietic cancer death was due to multiple myeloma. the authors suggested a possible link between benzene rind multiple myeloma.

A number of epidemiological studies have found significant mortality excess from leukaemia among workers exposed to solvents in the rubber industry. However. the relationship between this observed leukaemia excess and. specifically. benzene is less clear. In one cohort. benzene was not the predominant solvent used and the authors were hesitant in ascribing to benzene the sole responsibility for the excess of leukaemia (Monson & Nakano, 1976). In another cohort of rubber uorkers. the excess risk was limited to lymphocytic leukaemia and was not associated with either monocytic or myelocytic leukaemia (McMichael *et al.*, 1975, 1976; Wolf *et al.*, 1981). However. workers in this cohort were exposed to other solvents as well as benzene. although the latter often occurs as a contaminant of other solvents (e.g., petroleum naphtha).

To further investigate in this latter cohort the relationship between the observed excess of lymphocytic leukaemia and exposure to various solvents. Arp *et al.* (1983) examined the soltent exposure histories of 15 cases of lymphocytic leukaemia and 30 matched controls within this second cohort. An individual was classified as exposed to a solvent if the cumulative length of exposure exceeded 12 months. The relative risk estimates sere elevated for exposure to benzene (RR = 4.50) and to other solvents (RR = 4.50). Seither finding reached statistical significance at the 0.05 level. A much more detailed analysis of this same population was reported by Wilcosky *et al.* (1984). Details of estimated individual exposure to each of 25 solvents were obtained. Lymphosarcoma and lymphatic leukaemia were strongly associated with exposure to

carbon tetrachloride and carbon disulphide. whereas their association with benzene and xylene was of marginal statistical significance only.

Several epidemiological studies of petroleum refinery workers who are potentially exposed to petroleum products. including benzene. have also found an excess of lympho-haematopoietic cancers (Tabershaw Cooper Associates. 1974; Thomas *et al.*, 1982; Hanis *et al.*, 1982). However. none of these refinery studies provided any direct link between the observed mortality excess in lympho-haematopoietic cancer and benzene exposure.

Evaluation of the epidemiological evidence

On the basis of these studies there is substantial epidemiological evidence that persons exposed to benzene (or an associated impurity) experience an elevated risk of certain types of lymphatic and haematopoieric cancer. parricularly leukaemia. Reports providing such epidemiological evidence include those of **Aksoy** *et al.* (1974). Ott *et al.* (1978). Vianna and Polan (1979). Aksoy (1980). Rinsky *et al.* (1981). Rushton and Alderson (1981b). Decouflé *er al.* (1983) and Wong (1983). The most frequently reported form of leukaemia associated with benzene exposure is the acute myelocytic form. However. research on rubber workers (McMichael *et al.*, 1975. 1976) indicates that. in chronic low-level exposure situations. lymphocytic leukaemia may predominate. Perhaps this is a more likely manifestation of non-acute bone marrow insult. (Chronic forms of leukaemia are also less **likely** to be reported on death certificates as the underlying cause of death.)

On the other hand. there are reports indicating no increased risk of lympho-haematopoietic cancer as a result of benzene exposure. A recent cohort study (Tsai *et al.*, 1983) of **454** male refiner? workers exposed to benzene during 1952 to 1978 at a Texas refinery did not find any lympho-haematopoietic cancer deaths: however. the cohort size was small and the statistical power to detect a modest **risk** in either lympho-haematopoietic cancer or leukaemia was limited. The case-control studies of lymphocytic leukaemia among rubber workers (Arp *et ai.*, 1983; Wilcosky *et al.*, 1984) indicated that the lymphocytic leukaemia excess in the rubber industry came primarily from carbon tetrachloride and carbon disulfide. rather than from xylene or. even less likely. from benzene. The large mortality study of Thorpe (1974) of 38 000 petroleum uorkers potentially exposed to benzene has often been cited as showing no evidence of significant leukaemia excess as a result of exposure to benzene. This interpretation. however. ignores the comparison of the exposed workers with the control workers (from the same facilities. but not exposed to benzene): the exposed group had a two-fold risk for leukaemia relative to the non-exposed group.

There are a number of outstanding issues regarding the relationship between exposure to benzene and increased risk of lympho-haematopoietic cancers. First. none of the published studies provides adequate exposure inforniation for satisfactory dose-response analysis. Second. the choice of an appropriate comparison is an important issue. In a number of studies. analyses based on comparisons with the general population failed to detect an increased risk for lympho-haematopoietic cancer or leukaemia. In the Thorpe (1974) study in Europe. the industry-wide study of refiner), workers in the U.S.A. (Tabershaw Cooper Associates. 1974) and the

Table 4 Studies of chromosomal breakage in workers exposed lo "low' atmospheric concentrations of benzene

Author	Level of exposure to benzene (TWA)			
Picciano[a] (1979)	Controls	1 ppm	1–2 5 ppm	2 5–10 ppm
	3% (workers)[b]	22%	2646	33%
Sarto *et al* [c] (1984)		Range of snort-term samples = 0 2–12 4 ppm		
	12% (cells)[b]		2 7%	

[a]Benzene measured in 8-h TWA s chromosome breakage = breaks dicentrics translocations anc exchange figures
[b]% = % of workers with chromosome breakage
[c]Benzene measured in 5- an6 20-min samples

cohort study of eight oil refineries in Britain (Rush ton & Alderson, 1981a), the initial calculations of the leukaemia SMR. based on a comparison uith the general population. revealed no significant excess. However. subsequent analyses that rook account of variations in benzene exposure between categories of workers revealed positive associations with leukaemia. Third. although some studies (Aksoy. 1980: Ott *et ai..* 1978: Rinsky *et al.*. 1981) indicate that acute myelogenous leukaemia is the type frequently associated with benzene exposure. other reports show associations uith different leukaemia cell types (Tabershaw Cooper Associates. 1974: McMichael *et al.*, 1976: Linos *et al.*. 1980: Schottenfeld *et al.*. 1981). The studies of Decouflé *et al.* (1983) and Rinsky *et al.* (1985) have also suggested an increased risk of multiple myeloma.

Mutagenicity and chromosomal effects in humans

Numerous studies have been carried out on the chromosomes of bone-marrow cells and peripheral lymphocytes from people known to have been exposed to benzene (Dean. 1978; Picciano. 1979; Watanabe *et al.*, 1980: Sarto *et al.*, 1984). The populations included in these studies fall into two general categories: (1) patients with either a current or a past history of benzene-induced blood dyscrasias ("benzene haemopathies"). often associated with extensive exposure to benzene: and (2) workers with known current or past exposure to benzene. but with no obvious clinical effects. In some of these studies. significant increases in chromosomal aberrations have been seen: in some cases these have persisted for years after cessation of exposure (IARC, 1982).

The findings from two studies are summarized in Table 4. Picciano (1979) reported that 52 workers exposed to average benzene concentrations belou 10 ppm demonstrated a statistically significant two- to three-fold excess of chromosomal damage. compared to that observed among 44 unexposed workers. Furthermore. the percentage of uorkers exhibiting both chromosome breaks and marker chromosomes was 20. 25 and 32% for uorkers categorized as exposed to average benzene concentrations below 1. 1.0–2.5 and 2.5–10.0 ppni. respectively. Less than 3% of the unexposed workers exhibited both chromosome breaks and marker chromosomes More recently. Sarto *et al.* (1984) have reported supportive. albeit weaker. evidence of chromosomal breaks in workers esposed to benzene.

Other cancers in humans

Although induction of types of cancers other than of the lympho-haematopoietic system has been suspected. these have not been adequately evaluated from epidemiological studies of workers exposed to benzene. The studies have not had sufficient statistical power to detect low excess relative risks. However. since 1978. experimental studies have demonstrated that benzene administered either bv oral gavage or by inhalation induces cancer at multiple sites in experimental animal;. This research is reviewed further on.

Possible carcinogenicity of toluene and xylene

No epidemiological studies of differentiated exposure to toluene or xylene have been reported. It is possible that some or all of the above-mentioned increased cancer risk associated with non-specific exposure to solvents is attributable to these. or other. non-benzene solvents. However. evidence of any such effect will be difficult to obtain. particularly since benzene occurs as an impurity in toluene and xylene.

ANIMAL EXPERIMENTAL STUDIES OF BENZENE

Carcinogenicity

Intragastric administration of benzene dissolved in pure olive oil to female rats induced Zymbal gland carcinomas. mammary gland carcinomas and leukaemia. With the exception of leukaemias in the high-dose group. no such tumours occurred in male rats (Maltoni & Scarnato. 19791.

Experimental srudies of lifetime benzene inhalation at 300 ppm im mice show the widespread occurrence of anaemia. lymphocytopenia. neutrophilia and bone-marrow hypo- or hyperplasia (Snyder *et al.*. 1980). Statistically significant increases in the incidence of lympho-haematopoietic tumours (lymphocytic lymphoma. plasmacytoma and myelogenous leukaemia) also occurred (Snyder *et al.*. 1978a. 1980). However. no such tumour occurred in similarly exposed (male) rats (Snyder *et ai.*. 1978b).

Subsequent studies by Maltoni and co-workers haw shown that benzene is a potent carcinogen in rats of both sexes. whether ingested or inhaled: benzene produces different types of tumours in different organs and is therefore a "multipotential carcinogen" [Maltoni *et al.*. 1985).

Since all the benzene inhalation studies reported to date entail concentrations in the 200–300 ppm range. there is no direct animal experimental evidence relating to low-concentration exposures that now typify human occupational exposures. Long-term carcinogenicity bioassays at 50. 25. 10. 5 and 1 ppm may therefore be helpful for scientific risk assessment in humans.

Chromosomal damage

An earlier comprehensive review of the experimental data (IARC. 1982) concluded that benzene does not induce specific gene mutations in bacterial test systems. in

Drosophila melanogaster. or in mammalian cells. However. there was evidence that benzene induces cytogenetic abnormalities (chromosomal aberrations in bone-marrow cells) (IARC. 1982). Furthermore. the micronucleus test in mice and rats has been consistently positive. and numerous studies have shown that exposure of experimental animals to benzene in *vivo* leads to the induction of chromosomal aberrations in bone-marrow cells (IARC. 1982).

While Van Raalte *et al.* (1984) have argued that only **peak** exposure to benzene above 100 ppm can cause leukaemia or bone marrow toxicity. the results of recent epidemiological and experimental research suggest otherwise. Several recent animal experimental studies have demonstrated adverse effects on chromosomes and bone marrow as a result of low-level exposure to benzene.

It has recently been demonstrated by Tice *et al.* (unpublished data quoted by Infante & White. 1985) that exposure to benzene at a concentration of 10 pprn in air. *6* h dav for 9 days. induces a significant increase in micronuclei (a measure of bone-marrow chromosomal damage) in peripheral blood cells of mice. Mice exposed to 28 ppm benzene for a total of only 4 h had a two-fold increase in sister chromatid exchanges (SCE) in bone-marrow cells. The effect on mouse bone-marrow cells increased linearly with increasing levels of 4-h benzene exposure. indicating a linear dose-response. Previously. Kligerman had reported that mice exposed to 10 ppm benzene by inhalation for only *6* h demonstrated a significant increase in **SCE** in peripheral blood lymphocytes and in micronuclei in bone-marrow erythrocytes (Kligerman *et al.*. 1983).

Gad-El-Karim *et al.* (1984) have reported a dose-response increase in micronuclei in bone-marrou erythrocytes of mice administered 3 total of tuo oral doses of benzene at concentrations as low as 8.8 mg kg body weight. This dosage would be equivalent to two 8-h atmospheric exposures of approximately *6* ppm.

Collectively. these studies demonstrate chromosomal damage in bone-marrou cells as a result of the equivalent of one to several working day exposure to 10 ppm of benzene. **They** also demonstrate a linear dose-response for benzene exposure and chromosomal damage to both marrow and peripheral blood cells.

Since most leukaemias and related disorders in man seem to involve stem-cell anomalies. the response of haematopoietic stem cells was investigated by Baarson *et al.* (1984) who exposed mice by inhalation to 10 ppm benzene for 6 h day. 5 days week for 32. 66 and 178 days Progenitor cells from rhe esposed mice showed markedly reduced abilities to form colonies. compared to cells from control mice.

In another recent stud?. short-term exposure (6 h day for 6 days) to 10 ppm henzcne in air significantly depressed mitogen-induced blastogenesis of both B- and T-lymphocytes in mice (Rosen *et al.*. 1984). The authors concluded that benzene sxposure ai or near 10 ppm may affect immune function.

Thus. less than one week of exposure to 10 pprn of benzene is associated uith chromosomal damage to bone-marrow cells. significant depression of the bone marrow and disturbances of immune system function. Since most leukaemias and related disorders in man seem to involve stem-cell abnormalities and immune system deficiencies. these animal experimental findings may be highly relevant to leukaemia and exposure to benzene in humans. The findings provide further support for the conclusion that an elevated risk of a bone-marrow proliferative cancer

(leukaemia) could be expected as a result of exposure to 10 ppm benzene (Infante & White, 1985).

ANIMAL EXPERIMENTAL STUDIES OF TOLUENE ASD XYLENE

In a series of experiments. primarily directed at studying benzene carcinogenicity in rats. Maltoni *et al.* (1985) have also studied the effects of other benzene-correlated aromatics (toluene. xylene and ethyl-benzene). At high concentrations. toluene and xylene (and. to a lesser extent. ethyl-benzene) caused an increase in the total number of malignant tumours. The increase in tumour yield was approximately one-third that induced by an equal dose of benzene.

No other experimental data on this question appear to have been published.

SUMMARY

Benzene

The evidence for carcinogenicity of benzene in humans was evaluated by the **IARC** in **1982** as follows:

> "It is established that human exposure to commercial benzene or benzene-containing mixtures can cause damage to the haematopoietic system. including pancytopenia. The relationship between benzene exposure and the development of acute myelogenous leukaemia has been established in epidemiological studies.
>
> "Reports linking exposure to benzene with other malignancies were considered to **be** inadequate for evaluation.
>
> "There is *sufficient evidence* that benzene is carcinogenic to man."

This evaluation now warrants some elaboration and updating. While the epidemiological evidence concerning benzene carcinogenicity is strongest for acute myelocytic leukaemia. there **is some** limited evidence of increased risks of chronic myeloid and chronic lymphocytic leukaemia. In addition. recent studies have suggested an increased risk of multiple myeloma. while others indicate a dose-related increase for total lymphatic and hacmatopoietic neoplasms. Corroborative evidence for such a generalized effect comes from experimental studies showing that exposure to benzene dcprssses all lympho-haematopoietic cell lines.

While only limited evidence of benzene carcinogenicity in experimental animals exists. the recent findings of the National Toxicology Program (NTP. 1984) in the U.S.A. and Maltoni *et al.* (1985) strongly indicate that benzene is an experimental carcinogen.

Toluene and xylene

While no direct human etidence is available. there is recent evidence of carcinogenicity of toluene and xylene at high concentrations in experimental animals It should also be noted that any future epidemiological observations of cancer risks associated with toluene or xylene would have to take account of the suspected effects of benzene impurities

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APPENDIX (added in proof)

The extended follow-up of the NIOSH cohort (Rinsky *et al.*, 1985) has now been published (Rinsky *et al.*, 1987)[1]. These data appear to provide a better basis for quantitative risk assessment than do earlier studies. The authors estimated the cumulative exposure of individual workers to benzene by use of a job-exposure matrix to link detailed job histories with industrial hygiene measurement (historical air sampling of benzene concentrations).

The overall SMR for leukaemia (9 deaths) in benzene-exposed workers was 337 (95% confidence intenal, 154–641), and that for multiple myeloma (4 deaths) was 409 (110–1047).

For leukaemia, a dose-response relationship was evident, with SMRs increasing from 109 (2 cases) to 322 (2 cases), 1186 (2 cases) and 6637 (3 cases), with increases in cumulative benzene exposure from less than 40 ppm-years, to 40–199, 200–399 and 400 or more, respectively (400 ppm-years is equivalent to a mean annual exposure of 10 ppm over a 40-year working lifetime).

The authors concluded that an exponential function best described the dose-response relationship, and that a substantial decrease in the risk for death from leukaemia could therefore be achieved by lowering occupational exposure to benzene, including (importantly) exposure within the range 10 ppm–0.1 ppm.

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[1] Rinsky, R. A., Smith, A. B., Hornung, R., Filloon, T. G., Young, R. J., Okun, A. H. & Landrigan, P. J. (1987) Benzene and leukaemia: an epidemiologic risk assessment. *New Engl. J. Med.* *316*, 1044–1050.

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