

CHAPTER 1

CARCINOGENICITY OF BENZENE, TOLUENE AND XYLENE: EPIDEMIOLOGICAL AND EXPERIMENTAL EVIDENCE

A.J. McMichael

*Department of Community Medicine
University of Adelaide
Adelaide, South Australia*

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 exposure¹ 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.

¹Exposure concentrations are given throughout in parts per million - ppm. To convert to ISO recommended units, for benzene, 1 ppm = 3.25 mg/m³ (~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 have 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 100 000, 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 20-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 were too imprecise for further quantitative analysis.

Thorpe (1974) reported an epidemiological study of leukaemia incidence among workers from eight Esso Petroleum affiliates in Europe. Employees were divided into two groups: those with a potential exposure to 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) study of 748 white male workers exposed to benzene during the manufacture of rubber hydrochloride have been published on three occasions (Infante *et al.*, 1977, 1970; Rinsky *et al.*, 1981). The first two papers were based on the results with 75% complete follow-up of vital status, while 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 to 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 1000 workers exposed to benzene.

Number of years exposed	Exposure level			
	10 ppm		1 ppm	
	NIOSH study*	Dow study*	NIOSH study	Dow study
46 ^d	44-152	48-136	5-16	5-15
30	30-104	32-93	3-11	3-10
15	15-54	16-48	1.5-5	2-5
5	5-18	5-16	0.5-2	0.5-2
1	1-4	1-3	0.1-0.4	0.1-0.3

*From White *et al.* (1982).*Rinsky *et al.* (1981).*Ott *et al.* (1978).^d45 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 workers 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 1 000 workers exposed to benzene for an occupational lifetime.

Authors	Relative risk	Estimated cohort exposure	Excess risk per 1 000 workers	Excess risk per 1000 exposed to	
				10 ppm	1 ppm
Ott <i>et al.</i> (1978)	3.75	1-30 ppm for 8-9 years	72	24-720	2.4-72
Infante <i>et al.</i> (1977)	5.6	10-100 ppm, 8.5 years average	170	17-170	1.7-17
Rinsky <i>et al.</i> (1981)	21	10-100 ppm, ≥ 5 years exposure	140	14-140	1.4-14
Vigliani (1976)	20	200-500 ppm, 9 years average	475	9.5-23.8	1.0-2.4
Aksoy (1977)	25	150-210 ppm, 9.7 years average	534	25.4-35.6	2.5-3.6

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

inadequate for testing more complex models. Furthermore, as Infante and White (1985) 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 every increment of dose is accompanied by a commensurate increment in the excess cancer risk. The use of this toxicologically plausible model allows extrapolation of 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 and 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, with extrapolation to levels of 10 ppm and 1 ppm is shown in Table 2. The calculations in the last three columns were performed by Infante and White (1985), using the method applied by the IARC (1982) to the NIOSH study 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) of exposure to 10 ppm 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 time-weighted average (TWA) and a peak exposure level, whereas intermittently-exposed workers were characterized by a peak exposure level only. The eight-hour TWA's were 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 not 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 was** primarily due to 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 to benzene, and, specifically, leukaemia was of borderline significance when those exposed continuously and those exposed intermittently were grouped together, but the association between 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 continuously 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), while 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 1000 exposed persons"from animal inhalation studies'

Data set	Benzene air concentration (ppm)	Risk
All squamous-cell carcinomas, male rats, NTP (1984)	1 10	0.77 7.65
All squamous-cell carcinomas, male mice NTP (1984)	1 10	2.01 19.90
Leukaemia, both sexes, rats, Maltoni <i>et al.</i> (1983)	1 10	0.15 1.46
Leukaemia, male mice, Goldstein <i>et al.</i> (1982)	1 10	0.04 0.38

* Assuming risk to animals is the same as risk to humans when dose is measured in mg/kg body weight/day.

* Length of human exposure to benzene = 40 years.

* Table based on Crump and 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 years would attain a cumulative exposure of 400 ppm-years, which, in the Rinsky *et al.* (1981) cohort, would place him in a category for which 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 important role at lower exposure levels, and the estimates of risk are more uncertain. This is important, 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 (Decoufle *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 with 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.

Decoufle *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 were 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 and 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 workers, 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 solvent 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 were elevated for exposure to benzene ($RR = 4.50$) and to other solvents ($RR = 4.50$). Neither 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 haematopoietic cancer, particularly 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), Decouffé *et 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 refinery 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 al.*, 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 workers 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 information 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 refinery workers in the U.S.A. (Tabershaw/Cooper Associates, 1974) and the

Table 4. Studies of chromosomal breakage in workers exposed to "low" atmospheric concentrations of benzene

Author	Level of exposure to benzene (TWA)			
Picciano ^a (1979)	Controls 3% (workers) ^c	1 ppm 22%	1-2.5 ppm 26%	2.5-10 ppm 33%
Sarto <i>et al.</i> ^c (1984)	1.2% (cells) ^b	Range of short-term samples = 0.2-12.4 ppm 2.7%		

^a Benzene measured in 8-h TWA's; chromosome breakage = breaks, dicentric, translocations and exchange figures.

^b % = % of workers with chromosome breakage.

^c Benzene measured in 5 and 20-min samples.

cohort study of eight oil refineries in Britain (Rushton & Alderson, 1981a), the initial calculations of the leukaemia **SMR**, based on a comparison with the general population, revealed no significant excess. However, subsequent analyses that took account of variations in benzene exposure between categories of workers revealed positive associations with leukaemia. Third, although some studies (Aksoy, 1980; Ott *et al.*, 1978; Rinsky *et al.*, 1981) indicate that acute myelogenous leukaemia is the type frequently associated with benzene exposure, other reports show associations with different leukaemia cell types (Tabershaw/Cooper Associates, 1974; McMichael *et al.*, 1976; Linos *et al.*, 1980; Schottenfeld *et al.*, 1981). The studies of Decoufle *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 below 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 workers exhibiting both chromosome breaks and marker chromosomes was 20, 25 and 32% for workers categorized as exposed to average benzene concentrations below 1, 1.0-2.5 and 2.5-10.0 ppm, 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 exposed to benzene.

Oilier 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 by oral gavage or by inhalation induces cancer at multiple sites in experimental animals. 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, 1979).

Experimental studies of lifetime benzene inhalation at 300 ppm in 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 al.*, 1978b).

Subsequent studies by Maltoni and co-workers have 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 ppm in air, 6 h/day 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-Kariti *et al.* (1984) have reported a dose-response increase in micronuclei in bone-marrow erythrocytes of mice administered a total of two 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-marrow 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 the exposed mice showed markedly reduced abilities to form colonies, compared to cells from control mice.

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

Thus, less than one week of exposure to 10 ppm of benzene is associated with 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 AND 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 haematopoietic neoplasms. Corroborative evidence for such a generalized effect comes from experimental studies showing that exposure to benzene depresses 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 evidence 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,

REFERENCES

- Aksoy, M. (1977) Leukemia in workers due to occupational exposure to benzene. *New Istanbul Contrib. clin. Sci.*, 12, 3-14
- Aksoy, M. (1980) Different types of malignancies due to occupational exposure to benzene: a review of recent observations in Turkey. *Environ. Res.*, 23, 101-190
- Aksoy, M., Dincol, K., Erdem, S. & Dincol, G. (1972) Acute leukemia due to chronic exposure to benzene. *Am. J. Med.*, 52, 160-166
- Aksoy, M., Erdem, S. & Dincol, G. (1974) Leukemia in shoe-workers exposed chronically to benzene. *Blood*, 44, 837-841
- Arp, E.W., Wolf, P.H. & Checkoway, H. (1983) Lymphocytic leukaemia and exposures to benzene and other solvents in the rubber industry. *J. Occup Med.*, 25, 598-602
- Baaron, K.A., Snyder, C.A. & Albert, R.E. (1984) Repeated exposure of C57Bl mice to inhaled benzene at 10 ppm markedly depressed erythropoietic colony formation. *Toxicol. Lett.*, 20, 337-342
- Crump, K.S. & Allen, B.C. (1984) *Quantitative Estimates of Risk of Leukaemia from Occupational Exposure to Benzene*. Unpublished report prepared for U.S. Occupational Safety and Health Administration
- Dean, B.J. (1978) Genetic toxicology of benzene, toluene, xylene and phenols. *Mutat. Res.*, 47, 75-97
- Decoullé, P., Blattner, W. & Blair, A. (1983) Mortality among chemical workers exposed to benzene and other agents. *Environ. Res.*, 30, 16-25
- Delore, P. & Borgoniano, C. (1928) Acute leukemia following benzene poisoning. *J. Med. Lyon*, 9, 227-233
- Gad-El-Karim, M.M., Harper, B.L. & Legator, M.S. (1984) Modifications in the myeloclastogenic effect of benzene in mice with toluene, phenobarbital, 3-methylcholanthrene, Aroclor 1254 and SKF-525A. *Mutat. Res.*, 135, 225-243
- Goldstein, B. (1983) Benzene is still with us. *Atm. J. Ind. Med.*, 4, 585-587
- Goldstein, B.D., Snyder, C.A., Laskin, S., Bronberg, I., Albert, R.E. & Nelson, N. (1982) Myelogenous leukaemia in rodents inhaling benzene. *Toxicol. Lett.*, 13, 169-173
- Hanis, N.M., Holmes, T.M., Shallenberger, L.G. & Jones, K.E. (1982) Epidemiology study of refinery and chemical plant workers. *J. Occup. Med.*, 24, 203-212
- IARC (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 29, Some Industrial Chemicals and Dyestuffs*, Lyon, International Agency for Research on Cancer, pp. 93-148
- Infante, P. & White, M. (1985) Projections of leukemia risk associated with occupational exposure to benzene. *Am. J. Ind. Med.*, 7, 403-413
- Infante, P.F., Rinsky, R.A., Wagoner, J.K. & Young, R.J. (1977) Leukemia in benzene workers. *Lancet*, 2, 7618
- Infante, P.F., Rinsky, R.A., Wagoner, J.K. & Young, R.J. (1979) Leukemia in benzene workers. *J. Environ. Pathol. Toxicol.*, 2, 251-257
- Kligerman, A.D., Erexson, G.L. & Wilmer, J.L. (1983) Development of rodent peripheral blood lymphocyte culture systems to detect cytogenetic damage in vivo.

(Abstract) *Int. Symp. Sister Chromatid Exchange*, Brookhaven Natl. Lab., Dec. 1983

- Lincoln, A., Kyle, R.A., O'Fallon, W.M. & Kurland, L.T. (1980) A case-control study of occupational exposures and leukemia. *Int. J. Epidemiol.*, **9**, 131-135
- Maltoni, C. & Scarnato, C. (1979) First experimental demonstration of the carcinogenic effects of benzene. *Med. Lav.*, **5**, 352-357
- Maltoni, C., Conti, B., Cotti, G. & Belpoggi, F. (1983) A multipotential carcinogen. Results of long-term bioassays performed at the Bologna Institute of Oncology. *Am. J. Ind. Med.*, **4**, 589-630
- Maltoni, C., Conti, B., Cotti, G. & Belpoggi, F. (1985) Experimental studies on benzene carcinogenicity at the Bologna Institute of Oncology: current results and ongoing research. *Am. J. Ind. Med.*, **7**, 415-446
- McMichael, A.J., Spirtas, R., Kupper, L.L. & Gamble, J.E. (1975) Solvent exposure among rubber workers: an epidemiological study. *J. Occup. Med.*, **17**, 234-239
- McMichael, A.J., Spirtas, R., Gamble, J.R. & Tousey, P.M. (1976) Mortality among rubber-workers: relationship to specific jobs. *J. Occup. Med.*, **18**, 178-185
- Monson, R.R. & Nakano, K.K. (1976) Mortality among rubber workers. I. White male union employees in Akron, Ohio. *Am. J. Epidemiol.*, **103**, 284-296
- NTP (1984) National Toxicology Program. *Toxicology and carcinogenesis studies of benzene in F344/N rats and B6C3F mice (gavage studies)*. NTP-83-072, NIH Publication 84-2545. U.S. Department of Health and Human Services
- Ott, M.G., Townsend, J.C., Fishbeck, W.A. & Langner, R.A. (1978) Mortality among individuals occupationally exposed to benzene. *Arch. Environ. Health*, **33**, 3-9
- Picciano, D. (1979) Cytogenetic study of workers exposed to benzene. *Environ. Res.*, **19**, 33-38
- Rinsky, R.A., Young, R.J. & Smith, A.B. (1981) Leukemia in benzene workers. *Am. J. Ind. Med.*, **2**, 217-45
- Rinsky, R.A., Smith, A.B., Hornung, R., Filloon, T.G., Young, R.J., Okun, A.H. & Landrigan, P.J. (1985) *Benzene and leukemia: an epidemiologic risk assessment*. Unpublished report, U.S. Department of Health and Human Services, Public Health Service
- Rosen, M.G., Snyder, C.A. & Albert, R.E. (1984) Depressions in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. *Toxicol. Lett.*, **20**, 343-349
- Rushton, L. & Alderson, M.R. (1981a) An epidemiological survey of eight oil refineries in Britain. *Br. J. Ind. Med.*, **38**, 225-234
- Rushton, L. & Alderson, M.R. (1981b) A case-control study to investigate the association between exposure to benzene and deaths from leukemia in oil refinery workers. *Br. J. Cancer*, **43**, 77-84
- Sarto, F., Cominato, I., Pinton, A.M., Broveduni, P.G., Merler, E., Peruzzi, M., Bianchi, V. & Levis, A.G. (1984) A cytogenetic study of workers exposed to low concentrations of benzene. *Carcinogenesis*, **5**, 827-832
- Schottenfeld, D., Wnrshauer, M.E., Zaubler, A.G., Meikle, J.G. & Hart, B.R. (1981) A prospective study of morbidity and mortality in petroleum industry employees in the United States - a preliminary report. In: *Quantification of Occupational Cancer (Banbury Report 9)*. Cold Spring Harbor, NY, CSH Press, pp. 247-265

- Snyder, C.A., Goldstein, B.D., Sellakumar, A., Albert, R.E. & Laskin, S. (1978a) The toxicity of inhaled benzene (Abstract No. 106) *Toxicol. Appl. Pharmacol.*, 45, 265
- Snyder, C.A., Goldstein, B.D., Sellakumar, A., Wolman, S.R., Bromberg, I., Erlichman, M.N. & Laskin, S. (1978b) Hematotoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. *J. Toxicol. Environ. Health*, 4, 605-618
- Snyder, C.A., Goldstein, B.D., Sellakumar, A.R., Bromberg, I., Laskin, S. & Albert, R.E. (1980) The inhalation toxicology of benzene: incidence in hematopoietic neoplasms and hematotoxicity in AKR/J and C57BL/6J mice. *Toxicol. Appl. Pharmacol.*, 54, 323-31
- Tabershaw/Cooper Associates (1974) *A mortality study of petroleum refinery workers*. The American Petroleum Institute, Berkeley, California
- Thomas, T.L., Waxweiler, R.J., Moure-Erasso, R., Itaya, S. & Fraumeni, J.F., Jr. (1982) Mortality patterns among workers in three Texas oil refineries. *J. Occup. Med.*, 24, 135-141
- Thorpe, J.J. (1974) Epidemiologic survey of leukemia in persons potentially exposed to benzene. *J. Occup. Med.*, 16, 375-382
- Tsai, S.P., Wen, C.P., Weiss, N.S., Wong, O., McCellan, W.A. & Gibson, R.L. (1983) Retrospective mortality and medical surveillance studies of workers in benzene areas of refineries. *J. Occup. Med.*, 25, 685-692
- Van Raalte, H.G.S., Grasso P. & Irvine, D. (1984) Tackling a very difficult problem. Letter to the Editor. *Risk Anal.*, 4, 1-8
- Vianna, N.J. & Polan, A. (1979) Lymphomas and occupational benzene exposure. *Lancet*, 1394-1395
- Vigliani, E.C. (1976) Leukemia associated with benzene exposure. *Ann. N.Y. Acad. Sci.*, 271, 143-51
- Vigliani, E.C. & Saita, G. (1964) Benzene and leukemia. *New Eng. J. Med.*, 272, 872-876
- Watanabe, T., Endo, A., Kato, Y., Shima, S., Watanabe, T. & Ikeda, M. (1980) Cytogenetics and cytokinetics of cultured lymphocytes from benzene-exposed workers. *Int. Arch. Occup. Environ. Health*, 46, 31-41
- White, M., Infante, P. & Chu, K. (1982) A quantitative estimate of leukemia mortality associated with occupational exposure to benzene. *Risk Anal.*, 2, 195-203
- Wilcosky, T.C., Checkoway, H., Marshall, E.G. and Tyroler, H.A. (1984) Cancer mortality and solvent exposures in the rubber industry. *Am. Ind. Hyg. Assoc. J.*, 45, 809-811
- Wolf, P.H., Andjelkovich, D., Smith, A. & Tyroler, H. (1981) A case-control study of leukemia in the U.S. rubber industry. *J. Occup. Med.*, 23, 103-108
- Wong, O. (1983) *An industry-wide mortality study of chemical workers occupationally exposed to benzene*. Washington, D.C., Chemical Manufacturers Association, December, 1983
- WHO (1981) *Environmental Health Criteria 18, Arsenic*. Geneva, World Health Organization. pp. 144-145

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 interval, 154-641), and that for multiple myeloma (4 deaths) was 409 (10-1047).

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.

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