Chapter 5.2 Benzene

General description

Benzene (C_6H_6) is a colourless liquid at room temperature (melting point 5.5 °C) with a density of 0.87 g/cm^3 at 20 °C. It has a relatively low boiling point (80.1 °C) and a high vapour pressure (9.95 kPa at 20 °C), causing it to evaporate rapidly at room temperature. It is slightly soluble in water (1.8 g/litre at 25 °C) and miscible with most organic solvents.

Benzene in air exists predominately in the vapour phase, with residence times varying between a few hours and a few days, depending on the environment, the climate and the concentration of other pollutants. Reaction with hydroxy radicals is the most important means of degradation. It can also be removed from air by rain.

Sources

Benzene is a natural component of crude oil, and petrol contains 1–5% by volume. Within the European Union the maximum allowable concentration is 5%. Benzene is produced in large quantities from petroleum sources and is used for the chemical synthesis of ethyl benzene, phenol, cyclohexane and other substituted aromatic hydrocarbons. Production in 1988 was estimated to be 20 million tonnes worldwide and 5 million tonnes within the countries of the European Economic Community. Production in the USA and Japan in 1990 was estimated to be 5.4 million and 2.8 million tonnes, respectively *(1)*. Benzene is emitted during its production and from coke ovens. Besides these industrial sources, emission also occurs from different combustion sources, such as motor engines, wood combustion and stationary fossil fuel combustion. The major source is exhaust emissions and evaporation losses from motor vehicles, and evaporation losses during the handling, distribution and storage of petrol.

Occurrence in air

Daily median air concentrations in the USA have been reported as follows: remote areas, 0.51 µg/m^3 (0.16 ppb); rural areas, 1.50 µg/m^3 (0.47 ppb); and urban/suburban areas, 5.76 µg/m^3 (1.8 ppb) *(2)*. Mean concentrations from Montreal and Toronto in Canada, Houston, New York and Pittsburgh in the USA, Oslo in Norway, the Rhine area in Germany, London in England and Bilthoven in the Netherlands are in the range 2.8– 40 µg/m^3, with Bilthoven showing the lowest figure and Oslo the highest. Countrywide averages in Germany were further reported to be 1–10 µg/m^3. More recent data from ten Canadian cities surveyed between 1988 and 1990 have shown mean concentrations of benzene in the range 1.2–14.6 µg/m^3 with an overall mean value of 4.4 µg/m^3 *(3)*. Repeated weekly measurements during the winter of 1992–1993 in 17 towns in Sweden resulted in calculated winter mean levels in the range 3.3–10.4 µg/m^3. Similar measurements in 22 towns during the winter of 1993–1994 gave somewhat lower winter means of 2.6–7.4 µg/m^3 *(4)*. Mean values of benzene in "street canyons" in different German cities varied between 8 and 48 µg/m^3 *(5)*.

Higher concentrations than those reported above can occur in certain situations, such as during the refuelling of cars. Levels of 3.2 mg/m^3 (1 ppm) have been measured in the breathing zone during

refuelling *(2)*. Swedish measurements during refuelling have shown concentrations varying from 0.01 to 27 mg/m^3, depending on wind speed and local circumstances. The geometric mean value from 175 measurements was 0.76 mg/m^3. In comparison, 30 measurements during refuelling using a vapour recovery system showed a geometric mean value of 0.13 mg/m^3 *(6)*. The geometric mean personal exposure to benzene during a work shift for 32 petrol station attendants was 0.15 mg/m^3, with similar values in a station using a vapour recovery system *(7)*.

Cigarette smoke is an important source of benzene in indoor air, and median benzene levels have been found to be higher in the homes of smokers (10.5 µg/m^3) than those of nonsmokers (7 µg/m^3) in the USA. Corresponding figures from Germany were 11 and 6.5 µg/m^3, respectively. The levels in the USA were higher than the corresponding median outdoor concentration, 6 µg/m^3, and the mean personal exposure was also higher at 15 µg/m^3 *(2, 8)*. The mean concentration of benzene in indoor air in homes across Canada was 7.4 µg/m^3, with a maximum value of 68 µg/m^3. The mean concentration in outdoor air was 4.4 µg/m^3 *(3)*. Passive sampling in households in Germany (Duisburg) showed an average concentration of benzene in children's bedrooms of 9.5 µg/m^3 compared to 1.8 µg/m^3 outside the windows *(9)*. Indoor air concentrations are enhanced in dwellings near petrol stations *(10)*. Studies of benzene concentrations in the interior of vehicles while driving have shown values of 10–120 µg/m^3 in Germany, 37–57 µg/m^3 in Sweden, 30–115 µg/m^3 in the Netherlands, and mean values of 12–50 µg/m^3 in the USA *(5)*.

Conversion factors

1 ppm = 3.19 mg/m^3
1 mg/m^3 = 0.313 ppm

Analytical methods in air

Analytical methods for benzene in air usually include absorption traps and subsequent separation by gas chromatography with detection by flame ionisation, photo-ionization or mass chromatography. In recent years optical methods, such as differential optical absorption spectroscopy (DOAS), have also been applied. Unlike the other methods, which are point measurements, DOAS measures over a distance (500–1000 m) and records the mean concentration in the air in this path. However, the correlation between the two types of method is poor, DOAS usually giving higher values *(11)*.

Routes of exposure

Inhalation accounts for more than 99% of the exposure of the general population, whereas intake from food and water is minimal. Within the USA, the daily intake from ambient and indoor air has been calculated to range between 180 and 1300 µg/day, and the intake from food and water up to about 1.4 µg/day *(2)*. The average daily intake for an adult in Canada was estimated to be 14 µg from ambient air, 140 µg from indoor air, 1.4 µg each from food and drinking water and 49 µg from car-related activities, giving a total of 203 µg/day *(3)*. Wallace *(8)* estimated the corresponding average intake in the USA to be 320 µg/day. Cigarette smoking may add as much as 1800 µg/day and passive smoking 50 µg/day. Driving a car during the rush hour may give a significant intake. Assuming that 2 hours/day were spent in ambient air with a benzene concentration of 7 µg/m^3, 21 hours/day in indoor air at 4 µg/m^3, and 1 hour/day inside a vehicle at 50 µg/m^3, Fromme *(5)* calculated the relative uptakes from ambient air, indoor air, air inside cars, and intake from food to be 9%, 53%, 30% and 8%, respectively.

In a study carried out in Germany in 1990–1991 *(12)* with 113 persons selected at random over the country, the geometric mean of personal exposure to benzene was found to be 11 µg/m^3; the 95-percentile was 32 µg/m^3. Some 39% of the exposure could be explained, with 20% and 12% respectively being related to indoor exposure to environmental tobacco smoke and car-related activities (refuelling and time in transit).

Toxicokinetics

Absorption

Human inhalation studies at 160–320 mg/m^3 (50–100 ppm) suggest approximately 50% absorption and 30% retention of the inhaled dose, the rest being exhaled as unchanged benzene. Benzene is distributed throughout the body with lipid-rich and well perfused tissues containing the highest levels. Benzene can cross the placenta *(2)*.

Metabolism and elimination

The metabolism of benzene in animals and humans appears to be qualitatively similar. The oxidative metabolism occurs primarily in the liver through the cytochrome P-450 2E1 system. The main metabolites are phenol, catechol and hydroquinone. These compounds are also found in the bone marrow of laboratory animals. Phenol is the predominant metabolite in humans and is excreted in urine as sulfate and glucuronide conjugates. The formation of two of the toxic metabolites, benzoquinone and muconaldehyde, appears to be a saturable process. In mice, it has been shown that proportionally more benzene will be converted to toxic metabolites at low doses than at high doses. Furthermore, mice metabolize benzene faster and convert more of the benzene to toxic metabolites than rats. Because of this it has been suggested that metabolism in mice favours toxification pathways, while in rats it is primarily detoxification *(2)*.

The metabolism of benzene can be inhibited by toluene, leading to decreased toxicity. On the other hand, ethanol can increase the metabolism of benzene, primarily by inducing xenobiotic metabolizing enzymes *(13)*. The average half-time of benzene in humans is 28 hours *(14)*. In rats and mice, metabolites are excreted in the urine within 40 hours of dosing by any route of administration *(2)*.

Pharmacokinetic models have been developed for rats and mice and used for risk assessments based on animal cancer data *(2, 15)*. Physiologically based toxicokinetic models have also been developed using human data *(2, 16)*, but so far these have not been used for risk assessment.

Biomarkers of human exposure

At high exposure levels (above 32 mg/m^3, 10 ppm) there is a correlation between phenol excretion in urine and the level of exposure. At lower concentrations, overall benzene exposure is reflected in the amount excreted in breath *(14)*. Smokers have been found to exhale around 14 µg/m^3 and nonsmokers around 2 µg/m^3 *(8)*. The urinary excretion of the specific benzene metabolite *trans,trans*-muconic acid has been found to be enhanced in benzene-exposed workers and in smokers *(17–20)*. The excretion of 8-hydroxy-deoxyguanosine, formed as a result of oxidative DNA damage, correlated with benzene exposure in petrol station attendants *(21)*. Possible adducts with benzene oxide such as *N*-phenylvaline or *S*-phenylcysteine in haemoglobin could not be detected in benzene-exposed workers *(22)*, although there was a linear correlation of the latter adduct in albumin with benzene exposure (13–74 mg/m^3) in female Chinese workers *(18)*.

Health effects

Effects on experimental animals and *in vitro* test systems

The acute toxicity of benzene is low; LC_{50} values for mice and rats are 15 000 and 44 000 mg/m^3, respectively. Benzene is a general anaesthetic, causing depression of the central nervous system and loss of consciousness.

Haematotoxic effects

The myelotoxicity of benzene is well known. Decreases in haematological cell counts and in bone marrow cellularity have been demonstrated in several studies in mice after inhalation at a concentration of 320 mg/m^3 or higher for one or several weeks. Some effects have also been demonstrated at lower concentrations, and the lowest reported effect level for haematological effects is 32 mg/m^3 (10 ppm) for 25 weeks, at which concentration a depression in the numbers of circulating red cells and lymphocytes and in the number of splenic nucleated cells was observed *(2)*.

Rats seem to be less sensitive than mice. In one study, slight leukopenia occurred after inhalation of 150 mg/m^3 for 32 weeks, but two other studies with exposure during 2 or 13 weeks showed decreased leukocyte counts or decreased cellularity in bone marrow only at 960 mg/m^3 or higher *(2)*.

The myelotoxicity of benzene seems to be the result of the interaction between different benzene metabolites rather than the effect of a single one. It has been suggested that phenol stimulates the oxidation of hydroquinone to the reactive metabolite 1,4-benzoquinone by myeloperoxidase in the bone marrow *(2, 23)*. Stem cells, myeloid progenitor cells and stromal cells are sensitive targets *(24)*. A reduced production of colony-stimulating factors by stromal fibroblasts may be important *(25)*.

The toxicity of benzene is enhanced by ethanol. On the other hand, co-exposure to toluene reduced the degree of anaemia as observed in inhalation studies by Plappert et al. *(26)*.

Immunotoxic effects

Benzene-induced immunological effects are probably a reflection of bone marrow toxicity. A depression of the proliferative ability of B lymphocytes in mice has been demonstrated after 1 week of inhalation of benzene at concentrations down to 32 mg/m^3. T-cell response was depressed at 96 mg/m^3 *(2)*. Benzene reduced the host resistance to infection by *Listeria monocytogenes* in mice (960 mg/m^3 for 5 days) and increased the tumour incidence following injection of polyomavirus-induced tumour cells (320 mg/m^3 for 100 days) *(27)*. When mice were treated with daily oral administrations of benzene for 4 weeks, there was a reduction in peripheral blood lymphocytes, a biphasic splenic lymphocyte proliferative response to B- and T-cell mitogens, and a biphasic response for cell-mediated immunity: enhanced response at 8 mg/kg and a depression at 40 and 180 mg/kg *(2)*.

Reproductive effects

Benzene can cross the placenta of experimental animals, and haematopoietic changes have been observed in the fetuses and offspring of mice exposed to concentrations of 16–65 mg/m^3 during days 6–15 of gestation. Following exposure to high doses (400–1600 mg/m^3), decreased fetal

weight, an increase in fetal resorptions and skeletal variants have been found in the offspring of mice, rats and rabbits. However, no clear teratogenic effects have been demonstrated *(2)*.

Genotoxicity

Interaction with DNA. The benzene metabolites hydroquinone and benzoquinone form DNA adducts *in vitro (28, 29)*. DNA adducts were also observed *in vivo* by Bauer et al. *(30)* with the ^{32}P post-labelling method in liver cells of rabbits treated subcutaneously with benzene. However, Reddy et al. *(31, 32)* were unable to detect DNA adducts unequivocally following oral administration of benzene to rats and mice. Benzene metabolites, especially 1,2,4-benzenetriol, produce oxidative DNA damage forming 8-hydroxy-deoxyguanosine *in vitro*, and benzene forms the same adduct in the bone marrow of treated mice (200 mg/kg) *(33, 34)*. DNA damage, as examined with the single-cell gel assay for single-strand breaks, was detected in peripheral blood cells, bone marrow and liver in mice exposed to benzene at 900 mg/m^3 *(35)*.

Mutations in vitro. Negative results have been reported when testing benzene in the Ames *Salmonella/*microsome assay or other bacterial tests. However, Glatt et al. *(36)* found a slight mutagenic response in *Salmonella typhimurium* strain TA1535 when the assay was performed in desiccators rather than by plate incorporation.

The ring-opened metabolite of benzene, *trans,trans*-muconaldehyde, was weakly mutagenic in only one *Salmonella* strain (TA 104) but mutagenic in V79 hamster cells *(37)*. That study did not detect any mutagenic activity from muconic acid, but Rossman et al. *(38)* reported muconic acid to be mutagenic in *Escherichia coli*. Glatt et al. *(36)* investigated several other metabolites in *Salmonella* and V79 cells. Only 1,2-dihydrodiol, diolepoxides and 1,2,3-trihydroxybenzene were mutagenic in *Salmonella*; benzoquinone and hydroquinone gave the strongest mutagenic response in V79 hamster cells.

Chromosomal effects in vitro. There is some evidence that benzene can induce chromosomal abnormalities in mammalian cell cultures *(2)*. Metabolites of benzene (hydroquinone, catechol, diolepoxides and trihydroxybenzene) induced sister chromatid exchanges in V79 cells *(2, 36)*. Several metabolites, including muconaldehyde, have induced micronuclei in cell cultures *(34, 36, 39)*.

Mutations in vivo. Mice exposed to 0.13, 0.32 and 3.2 mg/m^3 for 22 hours/day, 7 days/week for 6 weeks were analysed for *hprt* mutations in spleen lymphocytes. There was a dose-related increase in mutant frequencies at 0.13 and 0.32 mg/m^3, but a decline at 3.2 mg/m^3 *(40)*. Transgenic mice have recently been used to test benzene *(41)*. *LacI* transgenic C57BL/6 mice exposed to 960 mg/m^3 for 12 weeks showed a significant increase in mutation frequency in lung and spleen tissue, but the increase was not significant in the liver.

Although benzene was negative in a sex-linked recessive lethal test in *Drosophila (42)*, exposure to 27 000 ppm by volume for 1 hour produced delayed mutations, scored as lethal in the third generation *(43)*.

Chromosomal effects in vivo. Benzene can induce structural and numerical chromosome aberrations, sister chromatid exchanges and micronuclei by various routes of exposure *(2)*. Most

studies were performed with fairly high concentrations, but Erexson et al. *(44)* detected sister chromatid exchanges in peripheral lymphocytes and micronuclei in the bone marrow of rats at 9.6 and 3.2 mg/m^3, respectively. Au et al. *(45)* were able to detect chromosome aberrations in lung macrophages after prolonged exposure (6 weeks) at concentrations as low as 0.32 mg/m^3, and in lymphocytes from the spleen of mice at 0.13 mg/m^3 *(46)*. However, there was no dose–response relationship in the latter study, as the highest exposure (3.2 mg/m^3) produced fewer aberrations than the middle exposure (32 mg/m^3).

Some studies have shown that benzene can cause transplacental cytogenetic effects in mice. An increased frequency of micronuclei in polychromatic erythrocytes of fetal liver and peripheral blood resulted from intraperitoneal injections of 220–880 mg/kg on day 14 of gestation, along with micronuclei in the dams *(47)*. Similar results were obtained by Xing et al. *(48)*, but not by Harper et al. *(49)*.

There has been no clear demonstration of dominant lethal effects in mice or rats. However, chromosome aberrations in spermatogonia and alterations in sperm head morphology have been demonstrated following intraperitoneal administration *(2)*.

The benzene metabolites hydroquinone and cathecol were weak inducers of micronuclei in the bone marrow of mice; phenol was negative. A combined administration of phenol and either cathecol or hydroquinone gave a synergistic response *(50, 51)*, probably through the formation of benzoquinone *(52)*. When hydroquinone was administered repeatedly for 3 days it induced both micronuclei and aneuploidy in mice *(53)*. Muconaldehyde induced sister chromatid exchanges but not micronuclei in mice after three repeated daily exposures *(2)*. Ciranni et al. *(54)* tested the transplacental effects of several metabolites; only hydroquinone gave rise to micronuclei in mice fetal cells.

Co-exposure to toluene alters the metabolism of benzene, and has been shown to reduce the extent of DNA damage caused by benzene inhalation *(26)*.

Carcinogenic effects

Benzene has been shown to be carcinogenic in mice and rats in several studies. Various types of lymphoma/leukaemia have been found, but the majority of neoplasms are of epithelial origin *(2)*.

In inhalation studies in mice, Cronkite and co-workers *(55–57)* used an exposure to 960 mg/m^3 for 16 weeks, followed by a lifetime observation period in order to avoid inhibition of cell replication. Lymphoma/leukaemia was found in C57BL/6 mice and myelogenous leukaemia but no lymphoma was found in CBA/Ca mice. Snyder et al. *(58)* exposed mice for lifetime to 960 mg/m^3. An increase was found in haematopoietic neoplasms, including thymic lymphoma, in C57BL/6J mice but not in AKR/J mice. C57BL/6 and CD-1 mice were exposed to the same concentration in a discontinuous exposure pattern (one week exposure, two weeks nonexposure) or to 3800 mg/m^3 for 10 weeks followed by a lifelong observation period. Neither of these exposures gave a significant increase in lymphoma/leukaemia, although other tumours such as lung adenomas and Zymbal gland carcinomas were induced *(59)*. An inhalation study with CBA mice exposed to 960 mg/m^3 for 16 weeks demonstrated an increase in malignant lymphomas, preputial gland carcinomas and lung adenomas, but not leukaemia *(60)*.

Inhalation studies with Sprague-Dawley rats have been performed by Snyder et al. *(61)* and Maltoni et al. *(62)*. There were few tumours in the animals, but increases in Zymbal gland carcinomas, mammary, liver and nasal tumours and leukaemia were reported at 320 and 640 mg/m^3.

Oral administration studies were performed by Maltoni et al. *(62)* and Huff et al. *(63)* with mice and rats, with daily doses ranging from 25 to 500 mg/kg body weight. The lowest dose of benzene that produced specific neoplasms varied from 25 mg/kg for adenomas of the lung, harderian gland and liver of mice to 500 mg/kg for lymphoreticular neoplasms in rats. Tumours were also induced in the Zymbal gland, oral and nasal cavities, mammary gland, forestomach, skin, preputial gland and ovary *(2)*.

Effects on humans

Haematotoxicity, bone marrow depression

Several types of blood dyscrasia, including pancytopenia, aplastic anaemia, thrombocytopenia, granulocytopenia and lymphocytopenia have been noted following exposure to benzene. As in experimental animals, the primary target organ for benzene that results in haematological changes is the bone marrow. It has been suggested that the cells at highest risk are the rapidly proliferating stem cells *(2)*.

Most of these cases were reported several years ago, when benzene was used as a solvent in different workplaces. An increased frequency of anaemia was detected among shoe workers, rotogravure workers and rubber factory workers with prolonged exposure to high benzene concentrations (hundreds of milligrams of benzene per m^3) *(2)*. When haematological surveillance data from rubber workers were analysed in retrospect, a decreased effect with time was observed in parallel with reduced levels of benzene in the workplace air from a mean of 240 mg/m^3 to 48–64 mg/m^3 *(64)*. Furthermore, it was found that the workers exposed above the median benzene exposure (approximately 120 mg/m^3) had significantly lower average white and red blood cell counts compared with workers exposed below the median concentration *(65)*. In a group of 200 workers exposed to 0.03–4.5 mg/m^3, no differences in haematological outcomes were observed compared to 268 nonexposed workers *(66)*. In an evaluation of the literature, a WHO Task Group *(2)* drew the conclusion that bone marrow depression or anaemia would not be expected to occur in workers exposed for 10 years to 3.2 mg/m^3 (1 ppm) or less.

Immunological effects

Older studies on workers exposed to benzene, toluene and xylene have shown decreased levels of agglutinins and IgG and IgA immunoglobulins, and increased levels of IgM. A loss of leukocytes has been observed in several studies in highly exposed workers as well as reduced numbers of T lymphocytes. In a study of refinery workers exposed to benzene concentrations of less than 32 mg/m^3, no such immunological effects were seen *(2)*.

Reproductive effects

Although benzene can cross the placenta, there is no evidence of teratogenic or other reproductive effects in humans, except a few older reports on disturbed menstrual cycle in women with high exposures to benzene and other aromatic solvents *(67)*. A recent study did not find any increased risk of spontaneous abortion among the wives of 823 male workers occupationally exposed to benzene levels of less than or around 15 mg/m^3 *(68)*.

Genotoxic effects

There are numerous studies on chromosomal effects in exposed workers *(2, 69)*. Both structural and numerical chromosome aberrations have been observed. In most cases the benzene exposure has also been high enough to produce haematological effects. The chromosomal effects in these studies are evident at concentrations of around 320 mg/m^3 (100 ppm) or higher, but in some studies effects were reported in workers chronically exposed to levels of around 32 mg/m^3 (10 ppm) *(2, 70, 71)*. Tompa et al. *(72)* reported that the frequency of chromosome aberrations decreased when exposure levels decreased from 3–69 mg/m^3 to 1–18 mg/m^3. In the study by Karacic et al. *(71)*, a decrease in sister chromatid exchanges but not in chromosomal aberrations was noted in a group of female workers when examined with a 5-year interval during which the mean benzene concentration had decreased from 26 to 16 mg/m^3. Smoking did not influence the results *(70, 71, 73)*.

Somatic mutations as an endpoint of benzene-induced genotoxic effects in heavily exposed workers was studied recently by Rothman et al. *(74)*. They used the glycophorin A (GPA) mutation assay. The results suggested that benzene induces gene-duplicating mutations, presumably through recombination mechanisms, but not gene-inactivating mutations due to point mutations or deletions.

Carcinogenic effects

Several clinical and epidemiological studies have shown that long-term exposure to benzene can lead to leukaemia, and benzene has been classified as a human carcinogen (Group 1) by IARC *(69)*.

One of the first epidemiological studies demonstrated an increased incidence of leukaemia, mainly acute myeloid leukaemia, in shoe workers in Istanbul *(75)*. Also shoe workers in Florence, Italy, showed an increased risk of leukaemia before 1963 *(76)*.

The most thorough and well investigated study, which has also been the main study used for quantitative risk assessment, is on workers that used to be employed in the manufacture of rubber film, the so-called Pliofilm cohort. The first report came out in 1977 *(77)*, and the study has been updated several times *(78–81)*. The cohort contains workers that were employed for at least 1 day during the years 1940–1965. Up to 1981, 9 cases of leukaemia and 4 cases of multiple myeloma had occurred among 1165 men (standardized mortality ratio (SMR) = 337 and 409, respectively). This is a statistically significant increase compared to the background incidence in the USA. In the update by Rinsky et al. *(79)*, a so-called nested case–control study was conducted within the cohort. Detailed exposure patterns were estimated for the 9 leukaemia cases and their 10 controls each. An exponential dose–response relationship between cumulative benzene exposure and leukaemia risk was found. According to this relationship, a worker occupationally exposed to an average of 3.2 mg/m^3 (1 ppm) for 40 years (40 ppm-years) would have an increased risk (odds ratio) of 1.7. Brett et al. *(82)* used the Rinsky model but made some minor changes in the choice of controls. Using earlier, higher exposure estimates than those used by Rinsky, they arrived at substantially lower risk estimates. Their estimate would lead to 0.5–1.6 excess cases of leukaemia among 1000 occupationally exposed workers, compared to 5.3 cases using the Rinsky exposure matrix (Table 1).

In an update of the Pliofilm cohort by Paxton et al. *(83)* the cohort had been expanded to include 1212 workers followed up to 1987. In total, 14 cases of leukaemia (8 of which were acute myelogenous or monocytic leukaemia) and 4 cases of multiple myeloma had occurred. The SMRs

for all lymphatic and haematopoietic cancers and for leukaemia were significantly increased (SMR = 221 and 360, respectively) but the SMR for multiple myeloma was no longer significantly increased. There was a strong trend in mortality from leukaemia with increasing cumulative exposure to benzene.

Another study used for risk assessment in the past was the Dow Chemical cohort *(84, 85)*, which revealed 4 cases of myelogenous leukaemia up to 1982 among 956 workers employed from 1938 onwards, a significantly increased number compared to the background incidence (SMR = 444). For leukaemia, however, there was no significantly increased risk (SMR = 194).

One large study from the USA comprised 7676 men employed in seven different chemical industries during 1946–1975, who were followed up to 1977 *(86)*. When the cancer incidence among the group of 3536 men continually exposed to benzene was compared to the incidence in the general population, the increased incidence of lympho-haematopoietic cancer was not statistically significant (SMR = 128). When a group of non-exposed workers was used as reference population, however, the risk for lympho-haematopoietic cancer was significantly increased (relative risk = 3.20). There were 7 cases of leukaemia in the exposed group and none in the reference group.

Table 1. Published leukaemia risk estimates for the Plioform cohort at two benzene exposure levels

Cases per 1000 workers exposed to:			
3.2 mg/m^3 (1 ppm)	**0.32 mg/m^3 (0.1 ppm)**	**Exposure matrix**	**Reference**
5.3	–	Rinsky et al. *(79)*	Brett et al. *(82)*
0.5–1.6	–	Rinsky et al. *(79)* Crump & Allen *(87)*	Brett et al. *(82)*
1.3	0.12	Rinsky et al. *(79)*	Paxton et al. *(81, 83)*
0.26	0.026	Crump & Allen *(87)*	Paxton et al. *(81, 83)*
0.49	0.048	Paustenbach et al. *(88)*	Paxton et al. *(81, 83)*

A study on 259 workers employed between 1947 and1960 in a chemical plant in the USA also gave a significant excess risk for lympho-haematopoietic cancer (SMR = 377). Three of the four deaths were from leukaemia, in two cases acute myeloid leukaemia *(89)*.

A large study from China encompassed 28 460 workers employed in different factories in 12 cities between 1972 and1981 *(90, 91)*. There were 30 cases of leukaemia (mainly acute forms) compared to 4 cases in a control population consisting of workers in other factories in the same cities (SMR = 574). There was also a significant increase in lung cancer (SMR = 231) *(90)*. In a follow-up, the study from China was expanded to include 74 828 benzene-exposed and 35 805 nonexposed workers employed for any length of time during 1972–1987 in 712 factories in 12 cities *(92, 93)*. Haematopoietic neoplasms and related disorders were identified in 82 patients in the exposed group, which was a significant increase compared to the control population (SMR = 340) *(94)*. Estimates of the exposure to benzene were highest for the rubber and plastics industry (100

mg/m^3), and for rubber glue applicators (170 mg/m^3) *(95)*. Detailed analysis of this large cohort, however, is not yet available.

Workers in petroleum refineries are potentially exposed to benzene and other hydrocarbons. Wong & Raabe *(96)* reviewed the literature up to 1989, which comprised several epidemiological studies worldwide, both published and unpublished. The data on lympho-haematopoietic cancers were inconsistent. Only 1 out of 15 cohort studies showed a significantly increased risk, but some studies indicated an increased mortality with length of employment. Concerning leukaemia, 6 out of 19 cohort studies showed a significantly increased risk. Overall, there was an increased total SMR for leukaemia of 110 (P = 0.1). Recent and updated studies on refinery and distribution workers have shown nonsignificant increased mortality from leukaemia in some groups of workers *(97–99)* and a statistically significant increase for road tanker drivers *(97)*. Follow-up studies that will quantify benzene exposures are not yet available.

For male workers in the petrol station industry, a significantly enhanced proportionate mortality ratio (PMR) for leukaemia (PMR = 328) was found between 1975 and 1985 *(100)*. When the risk of acute myeloid leukaemia was studied, using occupational information obtained from the Swedish 1970 census and follow-up in the Swedish Cancer Register from 1971 to 1984, an excess risk was found among male petrol station attendants *(101)*. No excess risk for leukaemia, however, was demonstrated among coke plant workers in general, or those specifically engaged in the production of benzene and other by-products *(102, 103)*.

Evaluation of human health risks

Exposure evaluation

Sources of benzene in ambient air include cigarette smoke, combustion and evaporation of benzene-containing petrol (up to 5% benzene), petrochemical industries, and combustion processes.

Mean ambient air concentrations of benzene in rural and urban areas are about 1 µg/m^3 and 5–20 µg/m^3, respectively. Indoor and outdoor air levels are higher near such sources of benzene emission as filling stations.

Inhalation is the dominant pathway for benzene exposure in humans. Smoking is a large source of personal exposure, while high short-term exposures can occur during refuelling of motor vehicles. Extended travel in motor vehicles with elevated air benzene levels (from combustion and evaporative emissions) produces exposures reported from various countries that are second only to smoking as contributors to the intensity of overall exposure. The contribution of this source to cumulative ambient benzene exposure and associated cancer risk comprises about 30% when the travel time is one hour, a duration not untypical for urban and suburban commuting by the general population.

Health risk evaluation

The most significant adverse effects from prolonged exposure to benzene are haematotoxicity, genotoxicity and carcinogenicity.

Chronic benzene exposure can result in bone marrow depression expressed as leukopenia, anaemia and/or thrombocytopenia, leading to pancytopenia and aplastic anaemia. Decreases in

haematological cell counts and in bone marrow cellularity have been demonstrated in mice after inhalation of concentrations as low as 32 mg/m^3 for 25 weeks. Rats are less sensitive than mice. In humans, haematological effects of varying severity have occurred in workers occupationally exposed to high levels of benzene. Decreased red and white blood cell counts have been reported above median levels of approximately 120 mg/m^3, but not at 0.03–4.5 mg/m^3. Below 32 mg/m^3, there is only weak evidence of effects.

The genotoxicity of benzene has been extensively studied. Benzene does not induce gene mutations in *in vitro* systems, but several studies have demonstrated induction of both numerical and structural chromosomal aberrations, sister chromatid exchanges and micronuclei in experimental animals and humans after *in vivo* benzene exposure. Some studies in humans have demonstrated chromosomal effects at mean workplace exposures as low as 4–7 mg/m^3. The *in vivo* data indicate that benzene is mutagenic.

The carcinogenicity of benzene has been established both in humans and in laboratory animals. An increased mortality from leukaemia has been demonstrated in workers occupationally exposed. Several types of tumour, primarily of epithelial origin, have been induced in mice and rats after oral exposure and inhalation exposure at 320–960 mg/m^3; these include tumours in the Zymbal gland, liver, mammary gland and nasal cavity. Lymphomas/leukaemias have also been observed, but with lower frequency. The results indicate that benzene is a multisite carcinogen.

Because benzene is characterized as a genotoxic carcinogen and recent data gathered in humans and mice suggest mutagenic potential *in vivo*, establishment of exposure duration and concentration in the human exposure studies is of major importance for the calculation of cancer risk estimates. The Pliofilm cohort is the most thoroughly studied. It was noted that significant exposures to other substances at the studied facilities were probably not a complicating factor, but that exposure estimates for this cohort vary considerably. Three different exposure matrices have been used to describe the Pliofilm cohort, i.e. those reported by Crump & Allen *(87)*, by Rinsky et al. *(79)*, and a newer and more extensive one by Paustenbach et al. *(88)*. The main difference between the first two is that the exposure estimates by Crump & Allen are greater for the early years, during the 1940s. Paustenbach et al. have, among other things, considered short-term, high-level exposure, background concentrations and absorption through the skin, which leads to exposure levels 3–5 times higher than those calculated by Rinsky et al. Compared to the Crump & Allen estimates, Paustenbach et al. arrived at higher exposure estimates for some job classifications, and lower ones for some others.

Within the most recently updated Pliofilm cohort, Paxton et al. *(81, 83)* conducted an extended regression analysis with exposure description for the 15 leukaemia cases and 650 controls. They used all three exposure matrices, which gave estimates of 0.26–1.3 excess cancer cases among 1000 workers at a benzene exposure of 3.2 mg/m^3 (1 ppm) for 40 years (Table 1).

Crump *(104)* calculated unit risk estimates for benzene using the most recently updated data for the Pliofilm cohort and a variety of models (Table 2). Multiplicative risk models were found to describe the cohort data better than additive risk models and cumulative exposure better than weighted exposures. Dose–responses were essentially linear when the Crump & Allen exposure matrix was used but, according to the author, there was evidence of concentration-dependent nonlinearity in

dose–responses derived using the Paustenbach et al. exposure matrix. In that case, the best fitting model was quadratic.

As can be seen in Table 2, the concentration-dependent model gives a much lower risk estimate than the other models when the Paustenbach exposure matrix is used. In such a model, the concentration of benzene is raised to the second power and thus given greater weight than the duration of exposure. Although there are biological arguments to support the use of a concentration-dependent model, many of the essential data are preliminary and need to be further developed and peer reviewed.

Table 2. Model-dependent worker risk and lifetime unit risk estimates for exposure to benzene for the Plioform cohort by Crump *(105)*[a]

Risk estimate	Linear	Nonlinear	Intensity-dependent	Exposure reference
Cases per 1000 workers exposed to 3.2 mg/m^3 (1 ppm)	5.1	5.0	5.1	Crump & Allen *(87)*
	3.8	2.9	0.036	Paustenbach et al. *(88)*
Unit risk per ppb	2.4×10^{-5}	2.4×10^{-5}	2.4×10^{-5}	Crump & Allen *(87)*
	1.5×10^{-5}	1.4×10^{-5}	1.7×10^{-10}	Paustenbach et al. *(88)*
Unit risk per µg/m^3 [b]	7.5×10^{-6}	7.5×10^{-6}	7.5×10^{-6}	Crump & Allen *(87)*
	4.7×10^{-6}	4.4×10^{-6}	5.3×10^{-11}	Paustenbach et al. *(88)*

[a] Multiplicative risk model, cumulative exposure.
[b] Calculated by converting ppb to µg/m^3.

Models giving equal weight to concentration and duration of exposure have been preferred here for the derivation of a risk estimate. Using multiplicative risk estimates and a cumulative exposure model, Crump *(105)* calculated a unit risk for lifetime exposure of $1.4–1.5 \times 10^{-5}$ per ppb with the Paustenbach exposure matrix, and of 2.4×10^{-5} per ppb with the Crump & Allen exposure matrix. If expressed in µg/m^3, the unit risk would thus range from 4.4×10^{-6} to 7.5×10^{-6}. With an additive model instead of a multiplicative model, the risk estimate would have been somewhat smaller. If similar linear extrapolations were done on the occupational cancer risk estimates by Paxton et al. (Table 1), unit risks lower by up to about one order of magnitude would result.

Guidelines

Benzene is carcinogenic to humans and no safe level of exposure can be recommended. For purposes of guideline derivation, it was decided to use the 1994 risk calculation of Crump rather than to derive new estimates. It was recognized that this use of existing analyses of the most recently updated cohort ruled out the inclusion of certain of the analyses noted earlier.

The geometric mean of the range of estimates of the excess lifetime risk of leukaemia at an air concentration of 1 µg/m^3 is 6×10^{-6}. The concentrations of airborne benzene associated with an excess lifetime risk of 1/10 000, 1/100 000 and 1/1 000 000 are 17, 1.7 and 0.17 µg/m^3, respectively.

References

1. EUROPEAN COMMISSION. *Environmental Chemicals Data Information Network (Ecdin)* (http://ulisse.etoit.eudra.org/Ecdin/Ecdin.html). Ispra, Italy, Joint Research Centre, 1993.
2. *Benzene*. Geneva, World Health Organization, 1993 (Environmental Health Criteria, No. 150).
3. HUGHES, K., MEEK, M.E. & BARTLETT, S. Benzene: Evaluation of risks to health from environmental exposure in Canada. *Environmental carcinogenesis and ecotoxicology reviews*, **C12**:161–171 (1994).
4. *Air quality in urban areas 1986–1994*. Stockholm, Statistics Sweden, 1994.
5. FROMME, H. Gesundheitliche Bedeutung der verkehrsbedingten Benzolbelastung der allgemeinen Bevölkerung [The significance of traffic-related benzene exposure for the general public]. *Zentralblatt für Hygiene*, **196**: 481–494 (1995).
6. NORDLINDER, R. & LJUNGKVIST, G. Benzene exposure at service stations, an occupational and environmental problem. *In*: Brown, R. H. et al., ed. *Clean air at work: new trends in assessment and measurement for the 1990s. Proceedings of an International Symposium, Luxembourg, 9–13 September 1991*. Cambridge, Royal Society of Chemistry, 1992 (Special Publication No. 108**),** pp. 93–95.
7. HARTLE, R. Exposure to methyl *tert*-butyl ether and benzene among service station attendants and operators. *Environmental health perspectives*, **101**: 23–26 (1993).
8. WALLACE, L.A. Major sources of benzene exposure. *Environmental health perspectives*, **82**: 165–169 (1989).
9. PORSTMANN, F. ET AL. Benzol und Toluol in Kinderzimmern [Benzene and toluene in children's rooms]. *Staub-Reinhaltung der Luft*, **54**: 147–153 (1994).
10. LAUE, W. ET AL. Untersuchungen über Benzolkonzentrationen in der Innenraumluft von Wohnungen in Nachbarschaft von Tankstellen [Investigation of benzene concentrations in the interior air of dwellings in the neighbourhood of petrol stations]. *Forum Städte-Hygiene*, **45**: 283–286 (1994).
11. BARREFORS, G. Monitoring of benzene, toluene and *p*-xylene in urban air with differential optical absorption spectroscopy technique. S*cience of the total environment*, **189**: 287–292 (1996).
12. HOFFMANN, K. ET AL. Umwelt-Survey 1990/91. Band IV: Personengebundene Exposition gegenüber flüchtigen organischen Verbindungen in den alten Bundesländern [Environment Survey 1990/91. Vol. IV: Personal exposure to volatile organic compounds in the "old" Federal States of Germany]. Berlin, Institut für Wasser-, Boden- und Lufthygiene, Umweltbundesamt, 1996 (WaBoLu-Hefte 4/96).
13. MEDINSKY, M.A., SCHLOSSER, P.M. & BOND, J.A. Critical issues in benzene toxicity and metabolism: the effect of interactions with other organic chemicals on risk assessment. *Environmental health perspectives*, **102**: 119–124 (1994).
14. *Air quality guidelines for Europe*. Copenhagen, World Health Organization Regional Office for Europe, 1987 (WHO Regional Publications, European Series, No. 23).
15. COX, L.A. & RICCI, P.F. Reassessing benzene cancer risks using internal doses. *Risk analysis*, **12**: 401–410 (1992).
16. WATANABE, K.H. ET AL. Benzene toxicokinetics in humans: exposure of bone marrow to metabolites. *Occupational and environmental medicine*, **51**: 414–420 (1994).

17. DUCOS, P. ET AL. *Trans,trans*-muconic acid, a reliable biological indicator for the detection of individual benzene exposure down to the ppm level. *International archives of occupational and environmental health*, **64**: 309–313 (1992).
18. BECHTOLD, W.E. & HENDERSON R.F. Biomarkers of human exposure to benzene. *Journal of toxicology and environmental health*, **40**: 377–386 (1993).
19. MELIKIAN, A.A., PRAHALAD, A.K. & SECKER-WALKER, R.H. Comparison of the levels of the urinary benzene metabolite trans,trans-muconic acid in smokers and nonsmokers, and the effects of pregnancy. *Cancer epidemiology, biomarkers and prevention*, **3**: 239–244 (1994).
20. POPP, W. ET AL. Concentrations of benzene in blood and *S*-phenylmercapturic and *t,t*-muconic acid in urine in car mechanics. *International archives of occupational and environmental health*, **66**: 1–6 (1994).
21. LAGORIO, S. ET AL. Exposure to benzene and urinary concentrations of 8-hydroxydeoxyguanosine, a biological marker of oxidative damage to DNA. *Occupational and environmental medicine*, **51**:739–743 (1994).
22. BADER, M., LEHNERT, G. & ANGERER, J. GC/MS determination of *N*-phenylvaline, a possible biomarker for benzene exposure in human hemoglobin by the "*N*-alkyl Edman method". *International archives of occupational and environmental health*, **65**: 411–414 (1994).
23. LEGATHE, A., HOENER, B.A. & TOZER, T.N. Pharmacokinetic interaction between benzene metabolites, phenol and hydroquinone, in B6C3F1 mice. *Toxicology and applied pharmacology*, **124**: 131–138 (1994).
24. SNYDER, R. ET AL. The toxicology of benzene. *Environmental health perspectives*, **100**: 293–306 (1993).
25. NICULESCU, R. & KALF G.F. A morphological analysis of the short-term effects of benzene on the development of the hematological cells in the bone marrow of mice and the effects of interleukin-1 alpha on the process. *Archives of toxicology*, **69**: 141–148 (1995).
26. PLAPPERT, U., BARTHEL, E. & SEIDEL, H.J. Reduction of benzene toxicity by toluene. *Environmental and molecular mutagenesis*, **24**: 283–292 (1994).
27. ROSENTHAL, G.J. & SNYDER, C.A. Inhaled benzene reduces aspects of cell-mediated tumor surveillance in mice. *Toxicology and applied pharmacology*, **88**: 35–43 (1987).
28. RUSHMORE, T. SNYDER, R. & KALF, G. Covalent binding of benzene and its metabolites to DNA in rabbit bone marrow mitochondria *in vitro*. *Chemical and biological interactions*, **49**: 133–154 (1984).
29. BODELL, W.J., LEVAY, G. & PONGRACZ, K. Investigation of benzene-DNA adducts and their detection in human bone marrow. *Environmental health perspectives*, **99**: 241–244 (1993).
30. BAUER, H., DIMITRIADIS, E.A. & SNYDER, R. An *in vivo* study of benzene metabolite DNA adduct formation in liver of male New Zealand rabbits. *Archives of toxicology*, **63**: 209–213 (1989).
31. REDDY, M.V. ET AL. ^{32}P analysis of DNA adducts in tissues of benzene-treated rats. *Environmental health perspectives*, **82**: 253–257 (1989).
32. REDDY, M.V. ET AL. Lack of DNA adduct formation in mice treated with benzene. *Mutation research*, **325**:149–155 (1994).

33. KOLACHANA , P. ET AL. Benzene and its phenolic metabolites produce oxidative DNA damage in HL60 cells *in vitro* and in the bone marrow *in vivo. Cancer research,* **53**: 1023–1026 (1993).
34. ZHANG, L. ET AL. Benzene metabolite, 1,2,4-benzenetriol, induces micronuclei and oxidative DNA damage in human lymphocytes and HL60 cells. *Environmental and molecular mutagenesis,* **21**: 339–348 (1993).
35. PLAPPERT, U. ET AL. Early effects of benzene exposure in mice. Hematological versus genotoxic effects. *Archives of toxicology,* **68**: 284–290 (1994).
36. GLATT, H. ET AL. Multiple activation pathways of benzene leading to products with varying genotoxic characteristics. *Environmental health perspectives,* **82**: 81–89 (1989).
37. GLATT, H. & WITZ, G. Studies on the induction of gene mutations in bacterial and mammalian cells by the ring-opened benzene metabolites *trans,trans*-muconaldehyde and *trans,trans*-muconic acid. *Mutagenesis,* **5**: 263–266 (1990).
38. ROSSMAN, T.G., KLEIN, C.B. & SNYDER, C.A. Mutagenic metabolites of benzene detected in the microscreen assay. *Environmental health perspectives,* **81**: 77–79 (1989).
39. YAGER, J.W. ET AL. Characterization of micronuclei induced in human lymphocytes by benzene metabolites. *Cancer research,* **50**: 393–399 (1990).
40. WARD, J.B.J. ET AL. The mutagenic effects of low level sub-acute inhalation exposure to benzene in CD-l mice. *Mutation research,* **268**: 49–57 (1992).
41. MULLIN, A.H. ET AL. Inhalation of benzene leads to an increase in the mutant frequencies of a *lacI* transgene in lung and spleen tissues of mice. *Mutation research,* **327**: 121–129 (1995).
42. KALE, P.G. & BAUM, J.W. Genetic effects of benzene in *Drosophila melanogaster* males. *Environmental mutagenesis,* **5**: 223–226 (1983).
43. KALE, P. & KALE, R. Induction of delayed mutations by benzene and ethylene dibromide in *Drosophila*. *Environmental and molecular mutagenesis,* **25**: 211–215 (1995).
44. EREXSON, G.L. ET AL. Induction of cytogenetic damage in rodents after short-term inhalation of benzene. *Environmental mutagenesis,* **8**: 29–40 (1986).
45. AU, W.W. ET AL. Development of a rodent lung macrophage chromosome aberration assay. *Mutation research,* **208**: 1–7 (1988).
46. AU, W.W. ET AL. Chromosome aberrations in lymphocytes of mice after sub-acute low-level inhalation exposure to benzene. *Mutation research,* **260**: 219–224 (1991).
47. NING, H. ET AL. Benzene-induced micronuclei formation in mouse fetal liver blood, peripheral blood, and maternal bone marrow cells. *Environmental and molecular mutagenesis,* **18**: 1–5 (1991).
48. XING, S.G. ET AL. Transplacental genotoxicity of triethylenemelamine, benzene, and vinblastine in mice. *Teratogenesis, carcinogenesis and mutagenesis,* **12**: 223–230 (1992).
49. HARPER, B.L., RAMANUJAM, V.M.S. & LEGATOR, M.S. Micronucleus formation by benzene, cyclophosphamide, benzo(*a*)pyrene, and benzidine in male, female, pregnant female, and fetal mice. *Teratogenesis, carcinogenesis and mutagenesis,* **9**: 239–252 (1989).
50. BARALE, R. ET AL. Genotoxicity of two metabolites of benzene: phenol and hydroquinone show strong synergistic effects *in vivo. Mutation research,* **244**: 15–20 (1990).
51. MARRAZZINI, A. ET AL. *In vivo* genotoxic interactions among three phenolic benzene metabolites. *Mutation research,* **341**: 29–46 (1994).
52. EASTMOND DA. Induction of micronuclei and aneuploidy by the quinone-forming agents benzene and *o*-phenylphenol. *Toxicology letters,* **67**: 105–118 (1993).

53. GUDI, R., XU, J. & THILAGAR, A. Assessment of the *in vivo* aneuploidy/micronucleus assay in mouse bone marrow cells with 16 chemicals. *Environmental and molecular mutagenesis*, **20**: 106–116 (1992).
54. CIRANNI, R. ET AL. Benzene and the genotoxicity of its metabolites. I. Transplacental activity in mouse fetuses and in their dams. *Mutation research*, **208**: 61–67 (1988).
55. CRONKITE, E.P. ET AL. Benzene inhalation produces leukaemia in mice. *Toxicology and applied pharmacology*, **75**: 358–361 (1984).
56. CRONKITE, E.P. ET AL. Benzene hematotoxicity and leukemogenesis. *American journal of industrial medicine*, **7**: 447–456 (1985).
57. CRONKITE, E.P. ET AL. Hematotoxicity and carcinogenicity of inhaled benzene. *Environmental health perspectives*, **82**: 97–108 (1989).
58. SNYDER, C.A. ET AL. The inhalation toxicology of benzene: Incidence of hematopoietic neoplasms and hematotoxicity in AKR/J and C57BL/6J mice. *Toxicology and applied pharmacology*, **54**: 323–331 (1980).
59. SNYDER, C.A. ET AL. The carcinogenicity of discontinuous inhaled benzene exposures in CD-1 and C57B1/6 mice. *Archives of toxicology*, **62**: 331–335 (1988).
60. FARRIS, G.M. ET AL. Carcinogenicity of inhaled benzene in CBA mice. *Fundamental and applied toxicology*, **20**: 503–507 (1993).
61. SNYDER, C.A. ET AL. Evidence for hematotoxicity and tumorigenesis in rats exposed to 100 ppm benzene. *American journal of industrial medicine*, **5**: 429–434 (1984).
62. MALTONI, C. ET AL. Benzene, an experimental multipotential carcinogen: Results of the long-term bioassays performed at the Bologna Institute of Oncology. *Environmental health perspectives*, **82**: 109–124 (1989).
63. HUFF, J.E. ET AL. Multiple-site carcinogenicity of benzene in Fischer 344 rats and B6C3F$_1$ mice. *Environmental health perspectives*, **82**: 125–163 (1989).
64. KIPEN, H.M. ET AL. Hematologic effects of benzene: A thirty-five year longitudinal study of rubber workers. *Toxicology and industrial health*, **4**: 411–430 (1988).
65. CODY, R.P., STRAWDERMAN, W.W. & KIPEN, H.M. Hematologic effects of benzene. Job-specific trends during the first year of employment among a cohort of benzene-exposed rubber workers. *Journal of occupational medicine*, **35**: 776–782 (1993).
66. COLLINS, J.J. ET AL. A study of the hematological effects of chronic low-level exposure to benzene. *Journal of occupational medicine*, **33:** 619–625 (1991).
67. *Toxicological profile for benzene*. Atlanta, GA, US Department of Health and Human Services, 1993.
68. STRUCKER, I. ET AL. Occupational paternal exposure to benzene and risk of spontaneous abortion. *Occupational and environmental medicine*, **51**: 475–478 (1994).
69. Benzene. In: *Some industrial chemicals and dyestuffs*. Lyon, International Agency for Research on Cancer, 1982, pp. 93–148 (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 29).
70. MAJOR, J. ET AL. Chromosome aberration, sister-chromatid exchange, proliferative rate index, and serum thiocyanate concentration in smokers exposed to low-dose benzene. *Environmental and molecular mutagenesis*, **23**: 137–142 (1994).
71. KARACIC, V. ET AL. Possible genotoxicity in low level benzene exposure. *American journal of industrial medicine*, **27**: 379–388 (1995).
72. TOMPA, A., MAJOR, J. & JAKAB, M.G. Monitoring of benzene-exposed workers for genotoxic effects of benzene: improved-working-condition-related decrease in the frequencies

of chromosomal aberrations in peripheral blood lymphocytes. *Mutation research,* **304**: 159–165 (1994).

73. TURKEL, B. & EGELI, U. Analysis of chromosomal aberrations in shoe workers exposed long term to benzene. *Occupational and environmental medicine,* **51**: 50–53 (1994).
74. ROTHMAN, N. ET AL. Benzene induce gene-duplicating but not gene-inactivating mutations at the glycophorin A locus in exposed humans. *Proceedings of the National Academy of Sciences,* **92**: 4069–4073 (1995).
75. AKSOY, M., ERDEM, S. & DICNOL, G. Leukaemia in shoe-workers exposed chronically to benzene. *Blood,* **44**: 837–841 (1974).
76. PACI, E. ET AL. Aplastic anemia, leukemia and other cancer mortality in a cohort of shoe workers exposed to benzene. *Scandinavian journal of work and environmental health,* **15**: 313–318 (1989).
77. INFANTE, P.F. ET AL. Leukaemia in benzene workers. *Lancet,* **2:** 76–78 (1977).
78. RINSKY, R.A., YOUNG, R.J. & SMITH, A.B. Leukaemia in benzene workers. *American journal of industrial medicine,* **2**: 217–245 (1981).
79. RINSKY, R.A. ET AL. Benzene and leukaemia. An epidemiologic risk assessment. *New England journal of medicine,* **316**: 1044–1050 (1987).
80. RINSKY, R.A. Benzene and leukaemia: An epidemiologic risk assessment. *Environmental health perspectives,* **82**: 189–191 (1989).
81. PAXTON, M.B. ET AL. Leukaemia risk associated with benzene exposure in the pliofilm cohort: I. Mortality update and exposure distribution. *Risk analysis,* **14**: 147–154 (1994).
82. BRETT, S.M., RODRICKS, J.V. & CHINCHILLI, V.M. Review and update of leukaemia risk potentially associated with occupational exposure to benzene. *Environmental health perspectives,* **82**: 267–281 (1989).
83. PAXTON, M.B. ET AL. Leukaemia risk associated with benzene exposure in the pliofilm cohort. II. Risk estimates. *Risk analysis,* **14**: 155–161 (1994).
84. OTT, M.G. ET AL. Mortality among individuals occupationally exposed to benzene. *Archives of environmental health,* **33**: 3–10 (1978).
85. BOND, G.G. ET AL. An update of mortality among chemical workers exposed to benzene. *British journal of industrial medicine,* **43**: 685–691 (1986).
86. WONG, O. An industry wide mortality study of chemical workers occupationally exposed to benzene. II Dose response analyses. *British journal of industrial medicine,* **44**: 382–395 (1987).
87. CRUMP, K. & ALLEN, B. Quantitative estimates of risk of leukaemia from occupational exposure to benzene. Washington, DC, US Department of Labor, 1984 (OSHA Docket H-059b, Exhibit 152, Annex B).
88. PAUSTENBACH, D.J. ET AL. Reevaluation of benzene exposure for the pliofilm (rubberworker) cohort (1936–1976). *Journal of toxicology and environmental health,* **36**: 177–231 (1992).
89. DECOUFLÉ, P., BLATTNER, W.A. & BLAIR, A. Mortality among chemical workers exposed to benzene and other agents. *Environmental research,* **30**: 16–25 (1983).
90. YIN, S.-N. ET AL. Leukaemia in benzene workers: a retrospective cohort study. *British journal of industrial medicine,* **44**: 124–128 (1987).
91. YIN, S.-N. ET AL. A retrospective cohort study of leukaemia and other cancers in benzene workers. *Environmental health perspectives,* **82:** 207–213 (1989).

92. LI, G.L. ET AL. Gender differences in hematopoietic and lymphoproliferative disorders and other cancer risks by major occupational group among workers exposed to benzene in China. *Journal of occupational medicine,* **36**: 875–881 (1994).
93. YIN, S.N. ET AL. Cohort study among workers exposed to benzene in China: I. General methods and resources. *American journal of industrial medicine,* **26**: 383–400 (1994).
94. TRAVIS, L.B. ET AL. Hematopoietic malignancies and related disorders among benzene-exposed workers in China. *Leukaemia & lymphoma,* **14**: 91–102 (1994).
95. DOSEMECI, M. ET AL. Cohort study among workers exposed to benzene in China: II. Exposure assessment. *American journal of industrial medicine,* **26**: 401–411 (1994).
96. WONG, O. & RAABE, G.K. Critical review of cancer epidemiology in petroleum industry employees, with a quantitative meta-analysis by cancer site. *American journal of industrial medicine*, **15**: 283–310 (1989).
97. RUSHTON, L. A 39-year follow-up of the U.K. oil refinery and distribution center studies: Results for kidney cancer and leukaemia. *Environmental health perspectives,* **101**: 77–84 (1993).
98. SCHNATTER, A.R. ET AL. A retrospective mortality study among Canadian petroleum marketing and distribution workers. *Environmental health perspectives,* **101**: 85–99 (1993).
99. WONG, O., HARRIS, F. & SMITH, T.J. Health effects of gasoline exposure. II. Mortality patterns of distribution workers in the United States. *Environmental health perspectives,* **101**: 63–76 (1993).
100. SCHWARTZ, E. Proportionate mortality ratio analysis of automobile mechanics and gasoline service station workers in New Hampshire. *American journal of industrial medicine,* **12**: 91–99 (1987).
101. JAKOBSSON, R. ET AL. Acute myeloid leukaemia among petrol station attendants. *Archives of environmental health,* **48**: 255–259 (1993).
102. HURLEY, J.F., CHERRIE, J.W. & MACLAREN, W. Exposure to benzene and mortality from leukaemia: results from coke oven and other coal product workers. *British journal of industrial medicine,* **48**: 502–504 (1991).
103. SWAEN, G.M.H. ET AL. Mortality of coke plant workers in the Netherlands. *British journal of industrial medicine,* **48**: 130–135 (1991).
104. CRUMP, KS. Risk of benzene-induced leukaemia: A sensitivity analysis of the pliofilm cohort with additional follow-up and new exposure estimates. *Journal of toxicology and environmental health,* **42**: 219–242 (1994).

