A Health Guideline Value for benzene in contaminated soil

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A Health Guideline Value for benzene in contaminated soil

Jack Dempsey
Therapeutic Goods Administration

1 INTRODUCTION

The contaminated site assessment model described by the ANZECC/NHMRC (1992) consists of four major facets: data collection and evaluation, toxicity assessment, exposure assessment and the contaminated site risk characterisation. This paper addresses the second facet, the toxicity assessment. The outcome of this assessment for a carcinogen should be a Guideline Dose, which is defined as the average daily intake of a chemical which, for a life time, should not result in cancer, based on the information available at the time of the assessment.

Specifically then, the purpose of this paper is to investigate the feasibility of setting of a Guideline Dose or value for benzene in contaminated soils using the modified benchmark dose methodology recommended by the NHMRC (1999). The development of a guideline value does not usually involve consideration of social and economic issues. Guideline values are usually not associated with monitoring or reporting protocols and do not have a goal for compliance. However, once the guideline value has been set, it can be used to determine whether the levels of the chemical concerned present at a contaminated site pose a human health problem, and can provide guidance for an appropriate management plan. The Guideline Dose may be used in the development of health investigation levels, response levels and risk characterisation of human exposures to contaminants in soil. The guideline dose is not intended to be used as a standard, or a fixed level indicating that action to reduce the level of a chemical is required, but as a guideline for decision making in particular situations (NHMRC, 1999).

1.1 THE SOURCES OF BENZENE

At normal ambient temperatures benzene is a liquid, but it evaporates rapidly at room temperature and is highly flammable. It has a characteristic aromatic odour and is slightly soluble in water (1.5 grams/litre at 20^{o}C) but miscible with most other organic solvents. Although it occurs naturally in crude petroleum most atmospheric benzene comes from anthropogenic sources. It is produced in extremely large quantities worldwide (14.8 million tonnes) as a component of petrol or as a chemical intermediate. Benzene is a major raw material used extensively as a solvent in the chemical and drug industries, as a starting material and intermediate in the synthesis of numerous chemicals, and as a petrol additive. Approximately 80% of the benzene consumed is used to produce ethylbenzene (55%), cumene (21%), cyclohexane (14%), aniline (5%), and miscellaneous other compounds (5%)(9th Report on Carcinogens).

Benzene is highly volatile and emissions to the atmosphere result from petroleum refining, the coking of coal, and the production of various aromatic compounds. Benzene may reside in the atmosphere for up to several days before it is removed by chemical reactions or by rain, a process that can lead to surface and ground water contamination. Benzene can move freely from soil to groundwater where it is slowly degraded by

anaerobic bacteria (weeks/months), but it rapidly evaporates from surface water where it is also rapidly degraded by aerobic bacteria (hours). It does not appear to bio-accumulate in aquatic or terrestrial systems (NICNAS, 2001).

1.2 PUBLIC EXPOSURE TO BENZENE

Benzene has been the subject of a comprehensive recent review by NICNAS (2001). As identified in that review, while all the environmental media can contribute as indirect sources of public exposure to benzene, the most important medium is air. The primary routes of potential human exposure to benzene are inhalation and dermal contact, with the former being the dominant pathway, accounting for more than 99% of the total daily intake. Although benzene is not persistent, it is ubiquitous in the atmosphere because of its widespread sources of release. In Australia, measured levels of benzene in air range up to 77 ppb for outdoor urban environments, 25 ppb in residential buildings (Brown, 2000) and 343 ppb inside petrol-fuelled vehicles (Duffy & Nelson, 1997). The NICNAS report estimates that the average atmospheric concentration of benzene in a model Australian urban environment is 3.4 ppb, with indoor/in-vehicle air levels ranging from 4.9-48 ppb.

General population exposures to benzene are not correlated with industrial or vehicular emissions. Figures from the USA show that emission sources comprise 82% from cars, 14% from industry, 3% from personal and home activities, and 0.1% from cigarettes. In contrast benzene exposures of the general population comprise 40% from cigarettes, 18% each from personal activities and car exhaust, 16% from home sources, 5% from environmental tobacco smoke, and 3% from industry (ATSDR, 1997). Similarly, in Australia the major sources of public exposure to benzene are thought to be direct exposure from cigarette smoking, petrol and miscellaneous consumer products.

The NICNAS report estimates that, based on a value of 50 µg/cigarette, the daily benzene intake of an average smoker is increased by 0.89 mg per day. For an adult person this corresponds to the continuous inhalation of ambient air containing 12.5 ppb of benzene. Petrol in Australia has a benzene content of 1-5% v/v, and direct exposure to benzene vapours occurs in garages and undercover parking stations, during petrol pumping at self-service petrol stations and inside vehicles. Reported air levels in these locations vary widely depending on local circumstances, but are typically in the order of 10-100 ppb. Consumer products such as paints, primers, paint strippers, lubricants, abrasives, model and hobby glues and other consumer products may contain organic solvents which in turn may contain very low concentrations of benzene as an impurity. The contribution to public exposure to benzene from these latter sources has not been quantified, but is expected to be very low (NICNAS, 1999).

Drinking water is a potential source of benzene through contamination of surface water and ground water from removal of benzene from the air in rain, leakage of underground storage tanks, and leaching of land-fill sites. In the USA, the intake from drinking water has been estimated at 0.2 µg/day. However, to date, benzene has not been detected in drinking water in Australia (NHMRC, 1996).

Benzene does not accumulate in the food chain and hence food is an unlikely source of substantial benzene exposure except through equilibration with surrounding media or through migration from cooking utensils and food packaging materials (NICNAS, 1999). However, benzene does occur at low levels in fruits, fish, vegetables, nuts, dairy products, beverages, and eggs. There is no indication in the published or unpublished literature

that food is an important source of exposure to benzene, accounting for at most 2.5% of total daily intake (CONCAWE, 2000). Overall, water and food-borne benzene are a small contribution to the total daily intake in non-smoking adults which is estimated as being between 3 and 24 µg/kg body weight per day (IPCS,1993). The average daily benzene air intake of urban residents in the USA is estimated to be 180 to 1,300 µg (ATSDR,1997).

Soil contamination by benzene may occur through spillage and leaks from underground storage tanks (USTs), and these events may be of significant local concern. However, these occurrences do not appear to be a major source of ambient levels of benzene. Data from the National Pollutant Inventory indicate that the highest five releases to the soil from major facilities totalled less than 80 kg of benzene per annum throughout the country. It is probable that USTs at service stations make a much larger, but unquantified contribution. When measurements have been made to determine the extent of direct contamination by spillage or leakage, benzene levels in soils at five facilities using or producing benzene in USA were found to range from <2 to191 µg/kg (IPCS, 1993).

2 TOXICOLOGY ASSESSMENT

2.1 METABOLISM

Benzene is well absorbed by the inhalation and oral routes in all animal species tested. It is also absorbed through the skin, although in practice skin contact is unlikely to result in significant absorption because of the rapid evaporation of the chemical. In humans, the absorption of benzene by the inhalation route is maximal within minutes of exposure and subsequently declines to a constant level, with 20-80% of the inhaled dose being retained after short-term exposure to air levels in the order of 1-100 ppm. In the body, benzene accumulates in lipid-rich tissues such as the brain. It also reaches the liver, where it is first metabolised by CYP2E1- mediated hydroxylation of the aromatic ring. Subsequent oxidations take place in several organs and result in a series of polyphenols and, to a lesser extent, cleavage of the ring, with a variety of metabolites occurring in the urine within 2 h of exposure. Benzene is also eliminated unchanged with exhaled air, particularly at higher exposure levels that saturate the enzymes which convert it to water-soluble metabolites.

2.2 ACUTE TOXICITY

Benzene is not highly acutely toxic to experimental animals. The only consistently reported acute systemic effects are CNS depression and cardio-respiratory arrest. In rats, the median lethal dose is 810-9900 mg/kg by mouth and 13,700 ppm by 4-h inhalation. The reported lethal dose in humans is 20,000 ppm by inhalation for 5-10 min, or 125 mg/kg by ingestion. The most significant acute effects are irritation of the skin, eyes and respiratory system at benzene vapour concentrations >33 ppm and progressive CNS depression at concentrations >250 ppm. Topically, benzene appears to be irritating to the skin and eyes.

2.2.1 Repeat dose toxicity

By repeated exposure, the main manifestations of benzene toxicity are CNS depression, immunosuppression, bone marrow depression, degenerative lesions of the gonads, and fetal growth retardation.

2.2.2 Reproductive toxicity

Overall, the above studies indicate that benzene exposure may cause degenerative changes in the gonads of mice, whereas there is insufficient evidence of similar effects in other species. There was also epithelial hyperplasia in the ovaries of mice in the NTP (1986) 2-year oral bioassay. However, this is likely to represent a preneoplastic lesion as ovarian tumours occurred with a significant positive trend in this study and epithelial hyperplasia was found in other organs with neoplastic lesions, namely the Harderian gland and the lungs. Compound-related testicular atrophy or degeneration was observed in male mice exposed to 300 ppm benzene by inhalation. Ovarian atrophy was observed in mice at 25 mg/kg/day by mouth and cystic ovaries at 300 ppm by inhalation. In both sexes, these lesions occurred at dose levels that were associated with haematological effects, but not with mortality or other signs of generalised toxicity. The available data on reproductive capacity are inconclusive (NICNAS, 2001).

2.2.3 Developmental toxicity

In several studies in pregnant animals exposed to benzene by inhalation or ingestion, there was a small, but statistically significant decrease in fetal body weight and an increase in the incidence of minor skeletal abnormalities at dose levels at which there was no evidence of maternal toxicity. Major structural abnormalities and abortions only occurred at dose levels that also caused marked toxicity in the dams. As such, benzene can be characterised as fetotoxic, but not teratogenic. Based on adequately reported rat studies that found fetal effects in the absence of any signs of maternal toxicity, the inhalation NOAEL for fetal growth disturbances is 40 ppm (Coate *et al*, 1984), with a LOAEL of 100 ppm (Coate *et al*, 1984; Green *et al*, 1978). Reliable oral effect levels cannot be determined from the available data (NICNAS, 2001).

2.2.4 Immunotoxicity and haematotoxicity

In animal studies, the immunological manifestations of benzene toxicity are related to effects on bone marrow, resulting in changes to both humoral and cellular acquired immunity. Bone marrow depression (haematotoxicity, 'benzene poisoning') from exposure to benzene has been reported in all species examined, including mice, rats, guinea pigs, rabbits, pigs and humans. Its main manifestation is a reduction in the number of one or more of the formed elements of the blood (leukocytes, erythrocytes, platelets) and/or in haemoglobin, and/or an increase or decrease in erythrocyte size (Mean Cell Volume). In humans, several occupational studies indicate that the incidence and severity of bone marrow depression is related to recent or current exposure to benzene. It is not possible to estimate the average latency period from the available human data; however, animal studies indicate that abnormal blood counts may develop in less than a month (NICNAS, 2001).

2.2.5 Genotoxicity

The toxic effects of benzene on human genetic material have been investigated in numerous *in vivo* studies and are adequately summarised in ATSDR (1997), IARC (1987), IPCS (1993), USEPA (1998a), and NICNAS (1999).

In vitro tests with benzene itself have predominantly produced negative results in conventional *in vitro* gene mutation assays in bacteria and mammalian cell systems, with and without metabolic activation. *In vitro* assays for chromosome aberrations have also

generally been negative, unless special precautions were taken to prevent the evaporation of benzene from the test system (Randall *et al*, 1993). Likewise, conventional *in vitro* tests for DNA breaks, unscheduled DNA synthesis and DNA synthesis inhibition have produced inconsistent results. Benzene has also been shown to induce morphological transformation, gene mutations through base substitutions, and aneuploidy in Syrian hamster embryo cells. Benzene and its metabolites have produced positive results in the alkaline single cell gel electrophoresis (Comet) assay (Anderson *et al*, 1995). Benzene was consistently negative in the sex-linked recessive lethal test in *Drosophila melanogaster*.

There is ample evidence that benzene is genotoxic in a broad spectrum of *in vivo* tests in rodents, in which the chemical was administered by inhalation, oral gavage or parenteral injection. These include tests for SCE and MN induction in peripheral blood cells, bone marrow cells, fetal liver cells, lung fibroblasts (Ranaldi *et al*, 1998), and Zymbal gland cells (Angelsanto *et al*, 1996); gene mutations in LC, lung and spleen cells; chromosome aberrations in LC, bone marrow cells, spleen cells, and spermatogonia; and DNA adducts in nucleated blood and bone marrow cells. Furthermore, many of these effects have been shown to be mitigated by inhibitors of benzene metabolism and reproduced by benzene metabolites such as hydroquinone and 1,2,4-trihydroxybenzene.

2.3 HUMAN TOXICITY

Exposure to benzene can result in haematotoxicity, immunotoxicity and carcinogenicity in humans and animals. Haematotoxicity resulting from chronic benzene exposure can present as anaemia, aplastic anaemia, leukopaenia, lymphocytopaenia, thrombocytopaenia, or pancytopenia (Aksoy, 1989). The principal carcinogenic response to chronic benzene exposure in humans is leukaemia while other animals tend to produce solid tumours in specific organs. While the liver is the initial site for the biotransformation of benzene, hepatotoxicity is not a consequence of benzene exposure. However, a number of studies have shown that for benzene to produce haematotoxicity in animals it must first be metabolised by the liver (Andrews *et al*, 1977; Sammett *et al*, 1979). Subsequent accumulation of the major hepatic metabolites, phenol, hydroquinone and catechol, occurs in the bone marrow where they are known to persist for varying durations after exposure to benzene ceases (Rickert *et al*, 1979). These metabolites act in an additive or synergistic manner to disrupt a range of mechanisms that regulate blood stem cell division and maturation and cause other cell damage through a combination of genetic and non-genetic changes. Damaged cells are usually eliminated, but may on occasion possess activated oncogenes or have lost tumour suppressor genes, which could enable them to proliferate as clonal lines of leukaemic cells.

There are also observations showing an association between long-term benzene exposure and the risk for lymphoma (non-Hodgkin's Lymphoma and Multiple Myeloma), although the evidence is not as conclusive as it is for leukaemia. Furthermore, structural and numerical chromosome aberrations have been detected in peripheral blood cells of workers exposed to high benzene levels. No threshold has been established for the genotoxic and carcinogenic effects of benzene. However, the available epidemiological evidence shows that the risk of leukaemia increases with exposure and is significantly elevated at cumulative exposures >50 ppm-years.

The kinetics and metabolism of benzene are similar in men and women. There are no data on age-related differences. However, there are likely to be substantial variations in the sensitivity of an individual to the toxic effects of the chemical as both genetic

polymorphisms and lifestyle factors may modulate the expression or activity of several key enzymes.

2.4 CARCINOGENICITY

"The tragedy of benzene is that it has taken so long for science to be translated into protective action. Many thousands of workers and other persons in nations around the world have suffered unnecessarily and died prematurely while regulatory agencies, industry and the courts debated the carcinogenicity of benzene and argued about the need for protective regulation. In the current era of global proliferation of toxic chemicals and hazardous technologies, all who are involved in the production and use of benzene have a heavy responsibility and a duty to protect their workers and the general public against this highly toxic and carcinogenic compound. The debate over whether benzene is carcinogenic has long since ended, and controversy about the need to protect humans against benzene must not continue".

Dr. Philip Landrigan, Chair of Community Medicine at Mount Sinai Hospital, New York, Editor-in-Chief, American Journal of Industrial Medicine

2.5 CARCINOGENICITY STUDIES IN ANIMALS

The review by NICNAS (1999) lists 23 carcinogenicity tests that have been reported in the open literature. They include oral gavage studies in B6C3F1, RF/J and Swiss mice and F344, Sprague-Dawley and Wistar rats and inhalation studies in AKR, C57BL, CBA, CD-1 and HRS mice and Sprague-Dawley rats. The available carcinogenicity studies provide clear evidence of a causal relationship between benzene exposure and malignant neoplasms in mice and rats. The tissues most commonly involved are various glandular or non-glandular epithelia of the oral cavity, nasal cavity, lungs and skin. The incidence of lymphoma was increased in several studies, but only in mice where this is a common spontaneously occurring tumour type. A proven reproducible animal model for benzene-induced leukaemia is not available (NICNAS, 1999).

The inhalational studies available for assessment were either poorly reported or inadequate for the determination of dose-response relationships for other reasons, such as an insufficient number of animals or range of exposure levels (NICNAS, 1999).

Of the available repeated dose oral studies, only the US National Toxicology Program's 2-year bioassays in mice and rats have been conducted and reported in full compliance with GLP and other internationally recognised quality standards (NTP, 1986). In these studies, benzene administered by oral gavage induced leukopaenia and lymphocytopaenia and an increase in the incidence of malignant tumours at the lowest dose level tested, namely 25 mg/kg/day in male and female mice and female rats and 50 mg/kg/day in male rats. A detailed evaluation is provided below.

NTP studies (1986)

Two-year toxicology and carcinogenesis studies of benzene (greater than 99.7% pure) were conducted in groups of 50 F344/N rats and 50 B6C3F1 mice of each sex and for each dose. Doses of 0, 50, 100, or 200 mg/kg body weight benzene in corn oil (5 ml/kg) were administered by gavage to male rats, 5 days per week, for 103 weeks. Doses of 0, 25, 50, or 100 mg/kg benzene in corn oil were administered by gavage to female rats and to male and female mice for 103 weeks. Ten additional animals in each of the 16 groups were

killed at 12 months and necropsies were performed. Hematologic profiles were performed at 3-month intervals.

In these 2-year studies, mean body weights of the 200 mg/kg male rats (-23%) and the 100 mg/kg mice (-14% to -19%) were lower than those of the vehicle controls, and survival of dosed groups decreased with increasing dose (rats--male: vehicle control, 32/50; low dose, 29/50; mid dose, 25/50; high dose, 16/50; female: 46/50; 38/50; 34/50; 25/50; mice--male: 28/50; 23/50; 18/50; 7/50; female: 30/50; 26/50; 24/50; 18/50). At week 92 for rats and week 91 for mice, survival was greater than 60% in all groups; most of the dosed animals that died before week 103 had neoplasia.

Compound-related non-neoplastic or neoplastic effects on the haematopoietic system, Zymbal gland, forestomach, and adrenal gland were found both for rats and mice. Further, the oral cavity was affected in rats, and the lung, liver, harderian gland, preputial gland, ovary, and mammary gland were affected in mice. Significantly increased ($P<0.05$) incidences of neoplasms were observed at multiple sites for male and female rats and for male and female mice. Primary neoplasms observed in rats and mice are summarised in Table 1.

Table 1 – tumours for which there is "clear evidence" of carcinogenicity

Species	Tumour	Sex	Incidence
Rat	Zymbal gland carcinomas	M	2/32, 6/46, 10/42, 17/42
		F	0/45, 5/40, 5/44, 14/46
	Oral cavity – papillomas and carcinomas combined	M	1/50 9/50 16/50 19/50
		F	1/50 5/50 12/50 9/50
	Skin - papillomas and carcinomas combined	M	0/50, 7/50, 4/50, 13/50
Mice	Zymbal gland carcinomas	M	0/43 1/34 4/40 21/39
		F	0/43 0/32 1/37 3/31
	Malignant lymphomas	M	4/49 9/48 9/50 15/49
		F	15/49 24/45 24/50 20/49
	Lung – adenomas and carcinomas combined	M	10/49 16/48 19/50 21/49
		F	4/49 5/42 10/50 13/49
	Harderian gland: adenoma	M	0/49 9/46 13/49 11/48
	Preputial gland: squamous cell carcinoma	M	0/21 3/28 18/29 28/35
	Ovary – benign mixed tumours	F	0/47 1/44 12/49 7/48
	Ovary – granulosa cell tumour	F	1/47 1/44 6/49 7/48
	Mammary gland - carcinomas	F	0/47 2/44 5/49 10/48

Dose levels: male rats - 0, 50, 100, 200 mg/kg; female rats and male and female mice - 0, 25, 50, 100mg/kg

The haematopoietic system of rats and mice of each sex was affected by benzene in the 2-year studies. The incidences of malignant lymphomas in all dosed groups of mice were greater than those in the vehicle controls (male: 4/49; 9/48; 9/50; 15/49; female: 15/49; 24/45; 24/50; 20/49). Lymphoid depletion of the splenic follicles (rats) and thymus (male rats) was observed at increased incidences. Bone marrow haematopoietic hyperplasia was observed at increased incidences in dosed mice of each sex (male: 0/49; 11/48; 10/50; 25/49; female: 3/49; 14/45; 8/50; 13/49).

The incidences of Zymbal gland carcinomas in mid and high dose male rats and in dosed female rats were greater than those in the vehicle controls (male: 2/32; 6/46; 10/42; 17/42; female: 0/45; 5/40; 5/44; 14/46). The incidences of Zymbal gland carcinomas in mid and high dose male mice and in high dose female mice were greater than those in the vehicle controls (male: 0/43; 1/34; 4/40; 21/39; female: 0/43; 0/32; 1/37; 3/31). In mid and high dose male mice and in high dose female mice, the incidences of epithelial hyperplasia of the Zymbal gland were also increased (male: 0/43; 3/34; 12/40; 10/39; female: 1/43; 1/32; 2/37; 6/31).

Hyperplasia of the adrenal capsule was observed at increased incidences in dosed mice of each sex (male: 2/47; 32/48; 14/49; 4/46; female: 5/49; 19/44; 34/50; 30/48). The incidence of pheochromocytomas in mid dose male mice was greater than that in the vehicle controls (male: 1/47; 1/48; 7/49; 1/46), whereas the incidences in dosed female mice were lower than that in the vehicle controls (female: 6/49; 1/44; 1/50; 1/48). Hyperplasia of the zona fasciculata of the adrenal cortex was observed at increased incidences in low dose rats of each sex (male: 0/50; 13/49; 0/48; 2/49; female: 0/50; 17/50; 0/47; 0/49).

Benzene was associated with increased incidences of neoplasms of the skin and oral cavity of rats. The incidences of squamous cell papillomas and squamous cell carcinomas of the skin in high dose male rats were greater than those in the vehicle controls (squamous cell papilloma: 0/50; 2/50; 1/50; 5/50; squamous cell carcinoma: 0/50; 5/50; 3/50; 8/50). Increased incidences of uncommon squamous cell papillomas or squamous cell carcinomas (combined) of the oral cavity were observed in dosed male and female rats (male: 1/50; 9/50; 16/50; 19/50; female: 1/50; 5/50; 12/50; 9/50). Incidences of squamous cell papillomas or carcinomas (combined) (male: 2/45; 2/42; 3/44; 5/38; female: 1/42; 3/40; 6/45; 5/42), hyperkeratosis, and epithelial hyperplasia of the fore-stomach were increased in some dosed groups of male and female mice; incidences of hyperkeratosis and acanthosis were increased in high dose male rats.

Compound-related effects in the lung, harderian gland, preputial gland, ovary, mammary gland, and liver were seen in mice but not in rats. Administration of benzene was associated with increased incidences of alveolar epithelial hyperplasia in mid and high dose mice (male: 2/49; 3/48; 7/50; 10/49; female: 1/49; 1/42; 9/50; 6/49). Increased incidences of alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas or carcinomas (combined) were observed in high dose male mice (carcinomas: 5/49; 11/48; 12/50; 14/49; adenomas or carcinomas: 10/49; 16/48; 19/50; 21/49). Alveolar/bronchiolar adenomas were seen at increased incidences in high dose female mice (4/49; 2/42; 5/50; 9/49), as were alveolar/bronchiolar carcinomas (0/49; 3/42; 6/50; 6/49) and alveolar/bronchiolar adenomas or carcinomas combined (4/49; 5/42; 10/50; 13/49) in mid and high dose female mice.

The incidences of focal or diffuse hyperplasia of the harderian gland were increased in dosed mice of each sex (male: 0/49; 5/46; 11/49; 7/48; female: 6/48; 10/44; 11/50; 10/47). The incidences of harderian gland adenomas (0/49; 9/46; 13/49; 11/48) in dosed male mice were greater than those in the vehicle controls. A marginal increase in the incidence of adenomas or carcinomas (combined) of the harderian gland was seen in high dose female mice (5/48; 6/44; 10/50; 10/47).

The administration of benzene to male mice was associated with increased incidences of hyperplasia (1/21; 18/28; 9/29; 1/35) and squamous cell carcinomas (0/21; 3/28; 18/29; 28/35) of the preputial gland. Increased incidences of mammary gland carcinomas were found in mid dose and high dose female mice (0/49; 2/45; 5/50; 10/49) and carcinosarcomas in high dose female mice (0/49; 0/45; 1/50; 4/49).

Increased incidences of various uncommon neoplastic and nonneoplastic lesions of the ovary (papillary cystadenoma, luteoma, granulosa cell tumor, tubular adenoma, benign mixed tumor, epithelial hyperplasia, and senile atrophy) were associated with the administration of benzene to female mice. In mid and high dose female mice, the incidences of granulosa cell tumours (1/47; 1/44; 6/49; 7/48) and benign mixed tumours (0/47; 1/44; 12/49; 7/48) were greater than those in the vehicle controls.

Increased incidences of hepatocellular adenomas were observed in low dose female mice (1/49; 8/44; 5/50; 4/49) and hepatocellular adenomas or carcinomas (combined) in low dose and mid dose female mice (4/49; 12/44; 13/50; 7/49).

2.6 CHOICE OF TUMOUR-TYPE FROM THE NTP STUDY

Some of the target organs in rodents such as the fore-stomach, harderian, Zymbal and preputial glands have no anatomical equivalent in humans. Moreover, human exposure to benzene is not associated with tumours of the mouth, nasal cavities or lung alveoli. These tumours were not analysed further.

There is some evidence of an elevated risk of malignant melanoma in humans exposed to benzene-containing products. However, in the NTP studies the increased incidence of epithelial skin tumours in male rats only occurred at the highest dose level tested (200 mg/kg/d), and this may have exceeded the maximum tolerated dose. These tumours were not analysed further.

There is some evidence of an elevated risk of breast cancer in humans exposed to benzene or benzene-containing products. Mammary gland carcinomas were increased in female mice at 50 and 100 mg/kg/day in the NTP study. The historical incidence of mammary gland carcinoma in this strain is approximately 1% (NTP, 1986). The incidence of these tumours has been used to derive a $BMD_{0.05}$.

The malignant lymphomas seen in the male and female mice are regarded as relevant to the haematopoietic tumours seen in humans. The incidence of these tumours has been used to derive a $BMD_{0.05}$

Calculation of $BMD_{0.05}$
NTP (1986) – male mice lymphomas $BMD_{0.05}$ = 16.64 mg/kg/d
NTP (1986) – female mice mammary carcinomas $BMD_{0.05}$ = 26.31 mg/kg/d

3 DERIVATION OF MODIFYING FACTORS

The next step in the modified-BMD method is the selection of the modifying factors, which encompass scientific uncertainty factors and safety factors. In accordance with the recommendations of the NHMRC (1999), the modifying factors for the NTP (1986) mouse tumours are proposed in Table 2, followed by justification for the factors chosen.

Table 2. Modifying factors to be applied to the benzene- $BMD_{0.05}$

Factors accounting for:	Range of factors recommended	Size of factor chosen
Interspecies extrapolation	<1-10	5
Intraspecies variability	1-10	10
Adequacy of data base	1-10	4
Seriousness of carcinogenic end-point:- Malignancy	1-10	10
Genotoxicity	1-5	5
Overall factor	<1-50,000	10,000

3.1 INTERSPECIES EXTRAPOLATION

The default modifying factor for animal to human extrapolation is 10. Benzene is relatively lipophilic and is metabolised to the much the same metabolites by the same pathways in all species studied. Although there is no good animal model for the haematopoetic effects seen in humans, the acutely toxic effects are similar in most species. A number of reviews of benzene toxicokinetics and metabolism are available, including IPCS (1993), ATSDR (1997), and NICNAS (2001). Urinalysis of several species exposed to benzene has demonstrated qualitative similarities in the spectrum of metabolites produced, indicating that the metabolism of benzene follows similar pathways between species including humans, and proceeds predominantly by hepatic CYP2E1-mediated oxidation of the aromatic ring to yield benzene oxide/oxepin. Several studies have confirmed that benzene accumulates in the adipose tissue, bone marrow and brain of animals and humans.

A factor of 5 is proposed. A smaller number might be considered since mice may be more sensitive than humans or rats. The easily saturated nature of benzene metabolic pathways and greater respiratory minute volume of the mouse allow the mouse to expire more of an oral dose of benzene compared to the rat. Similarly, respiratory differences and the greater metabolic rate of the mouse allow tissue levels of benzene metabolites to reach higher levels compared to the rat (NICNAS, 1999).

3.2 INTRA-SPECIES VARIABILITY

For intra-species variability, in cases where the modified-BMD is derived from animal data and if there were no human data on the variability of response to benzene, the default value of 10 would be used. However, in the case of benzene there is human data that demonstrates that the variation in benzene absorption between individuals following inhalation is high. Additionally the major metabolic pathway is dependent on CYP2E1 activities within the liver and these have been found to vary by more than ten-fold between individuals. The default value of 10 is chosen.

3.3 ADEQUACY OF THE DATA BASE

The findings from the sundry reviews of benzene toxicity indicate a reasonable confidence in the quality of the data base and the degree of confidence that can be attached to the derived modified-BMD. It should be noted that human studies are relatively few and generally restricted to OH&S circumstances where the quantitation of the doses is somewhat lacking. In accordance with the NHMRC guidelines, a medium degree of confidence can be placed in the database and a modifying factor in the range of 3-7 can be chosen. A modifying factor of 4 is proposed.

3.4 SERIOUSNESS OF CARCINOGENIC END-POINT:- MALIGNANCY

Under the conditions of the 2-year gavage studies, there was clear evidence of carcinogenicity of benzene for male F344/N rats, for female F344/N rats, for male B6C3F1 mice, and for female B6C3F1 mice. For male rats, benzene caused increased incidences of Zymbal gland carcinomas, squamous cell papillomas and squamous cell carcinomas of the oral cavity, and squamous cell papillomas and squamous cell carcinomas of the skin. For female rats, benzene caused increased incidences of Zymbal gland carcinomas and squamous cell papillomas and squamous cell carcinomas of the oral cavity.

For male mice, benzene caused increased incidences of Zymbal gland squamous cell carcinomas, malignant lymphomas, alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas or carcinomas (combined), harderian gland adenomas, and squamous cell carcinomas of the preputial gland. For female mice, benzene caused increased incidences of malignant lymphomas, ovarian granulosa cell tumours, ovarian benign mixed tumours, carcinomas and carcinosarcomas of the mammary gland, alveolar/bronchiolar adenomas, alveolar/bronchiolar carcinomas, and Zymbal gland squamous cell carcinomas.

The ability of benzene to induce malignancy in a range of tissues is well-established from many studies. On the suggested scale of 1-10 for malignancy, a modifying factor of 10 is proposed for the "Seriousness of the Carcinogenic End-point", reflecting a high degree of confidence in benzene's ability to induce malignant lymphomas and mammary gland tumours.

3.5 GENOTOXICITY

The proposed range of modifying factors for genotoxicity is 3-5 when the agent is considered likely to pose a genotoxic hazard. In view of the well-documented *in vivo* genotoxicity of benzene, a modifying factor of 5 is considered appropriate.

As shown in Table 2, the multiplication of these assigned figures results in a total modifying factor of 10,000.

Calculation of benzene guideline values
The next stage in generating a guideline value is to divide the modified-BMDs by the total modifying factor (10,000).

As the BMDs were:– male mice lymphomas BMD_{05} = 16.64 mg/kg/d, – female mice mammary carcinomas $BMD_{0.05}$ = 26.31 mg/kg/d, then the guideline dose is 1.7-2.6 µg/kg/d.

Guideline values from oral animal studies
Choosing the lowest animal-derived guideline dose of 1.7 µg/kg/d, in a 70 kg adult this equates to a daily benzene intake of 119 µg ; for a child (2.5 year old, 13.2 kg) the allowable intake is 20 µg .

4 HUMAN STUDIES

In several of the occupational cohort studies available for review, past employment in an industry or job category with the potential for exposure to benzene was associated with a significant increase in the risk for cancer of the blood and lymphatic system and/or

leukaemia. In addition, there was a significant trend with cumulative exposure, that is, a clear dose-time-response relationship in three out of four studies in which detailed exposure assessments were made. The critical study which has informed previous standard setting is the Pliofilm cohort.

In the largest cohort studied to date, the latency period from first exposure to clinical diagnosis was estimated at 11-12 years, with a range from 10 months to 50 years. This data is emerging from a very large cohort of nearly 80,000 benzene workers in China, supported by the US National Cancer Institute (Yin *et al*, 1996). The summary data from these two studies is presented in the table below.

Table 3 - Dose response for Acute Myeloid Leukaemia in the Pliofilm and Chinese cohorts

Pliofilm cohort				
Dose (ppm)	30	120	300	400
Cases	1	0	2	3
RR	1	0.1	20	100
Chinese cohort				
Dose (ppm)	0	30	50	100
Cases	4	7	7	14
RR	1	2.7	6	4.4

Calculation of $BMD_{0.05}$

Pliofilm cohort – 810 ppm years
Chinese cohort – 25 ppm years

Using the lowest value of 25 ppm years, and a working life of 40 years, then the benchmark dose TWA_8 is calculated to be 0.6 ppm.

Derivation of modifying factors

Table 4. Modifying factors to be applied to the benzene- $BMD_{0.05}$

Factors accounting for:	**Range of factors recommended**	**Size of factor chosen**
Interspecies extrapolation	<1-10	1
Intraspecies variability	1-10	10
Adequacy of data base	1-10	4
Seriousness of carcinogenic end-point:- Malignancy	1-10	10
Genotoxicity	1-5	5
Overall factor	<1-50,000	2000

4.1 INTERSPECIES EXTRAPOLATION

As human data have been used to derive the BMD no interspecies extrapolation is required. Factor chosen = 1.

4.2 INTRA-SPECIES VARIABILITY

For intra-species variability, there is human data that demonstrates that the variation in benzene absorption between individuals following inhalation is high. Additionally the major metabolic pathway is dependent on CYP2E1 activities within the liver and these have been found to vary by more than ten-fold between individuals. A factor of 10 is chosen.

4.3 ADEQUACY OF THE DATA BASE

The findings from the sundry reviews of benzene toxicity indicate a reasonable confidence in the quality of the data base and the degree of confidence that can be attached to the derived modified-BMD. It should be noted that human studies are relatively few and generally restricted to OH&S circumstances where the quantitation of the doses is somewhat lacking. In accordance with the NHMRC guidelines, a medium degree of confidence can be placed in the database and a modifying factor in the range of 3-7 can be chosen. A modifying factor of 4 is proposed.

4.4 SERIOUSNESS OF CARCINOGENIC END-POINT:- MALIGNANCY

The ability of benzene to induce malignancy in a range of tissues is well-established from many studies. On the suggested scale of 1-10 for malignancy, a modifying factor of 10 is proposed for the "Seriousness of the Carcinogenic End-point", reflecting a high degree of confidence in benzene's ability to induce leukaemias and possibly other tumours.

4.5 GENOTOXICITY

The proposed range of modifying factors for genotoxicity is 3-5 when the agent is considered likely to pose a genotoxic hazard. In view of the well-documented *in vivo* genotoxicity of benzene, a modifying factor of 5 is considered appropriate.

As shown in the Table, the multiplication of these assigned figures results in a total modifying factor of 2000.

5 CALCULATION OF BENZENE GUIDELINE VALUES

The next stage in generating a guideline value is to divide the modified-BMDs by the total modifying factor (2,000).

As the lowest modified $BMD_{0.05}$ was for the Chinese cohort = 25 ppm years giving a TWA_8 of 0.6 ppm, then the guideline dose is 0.3 ppb

6 SUMMARY

	Animal studies NTP (1986)	**Human studies (Chinese cohort)**	**USEPA, 1998**
Tumours 0	male mice lymphomas	AML	ALC
$BMD_{0.05}$ (TWA_8)	16.64 mg/kg/d	0.6 ppm	7.6 (NOAEL)
Modifying factor	10,000	2,000	1000
Guideline dose	1.7 µg/kg/d	0.3 ppb	3 ppb
Total daily intake.	adult 119 µg , child 20 µg		
	Canada	UK	IPCS (1993)
Tumours	AML	AML	

	Animal studies NTP (1986)	**Human studies (Chinese cohort)**	**USEPA, 1998**
$BMD_{0.05}$ (TWA_8)	4.6 ppm (TD_{05})	0.5 ppm (BMD_0)	1 ppm (BMD_0)
Modifying factor		100	
Guideline dose		5 ppb	
Total daily intake.			

The general population exposure levels were estimated to range from 1.19-12.80 ppb (IEH, 1999).

An estimate of long-term average exposures in the general population is 4.7 ppb (Wallace, 1996).

Notice: The modified-Benchmark Dose approach is still being appraised and developed. The values derived in this paper represent a single trial of the method and have not been endorsed by the NHMRC or the Contaminated Sites workshop. Final values are likely to be different from these proposed values.