RATIONALE FOR THE DEVELOPMENT OF

ONTARIO AIR STANDARDS

FOR

BENZENE

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Standards Development Branch
Ontario Ministry of the Environment

Executive Summary

The Ontario Ministry of the Environment (MOE) has identified the need to develop and/or update air quality standards for priority contaminants. The Ministry's Standards Plan, which was released in October, 1996 and revised in November, 1999, identified candidate substances for which current air standards will be reviewed or new standards developed. Benzene was identified as a high priority compound for review based on its pattern of use in Ontario and recent toxicological information. Once a decision is made on the air standards, they will be incorporated into Ontario Regulation 419: Air Pollution – Local Air Quality (O. Reg. 419/05). The Ambient Air Quality Criterion (AAQC) will be incorporated into Schedule 3 of the regulation and the half hour standards will be incorporated into Schedule 2. This rationale document provides a review of scientific and technical information relevant to the setting of Ambient Air Quality Criteria (AAQC) and Point of Impingement (POI) standards for benzene.

Benzene (C_6H_6) (CAS# 71-43-2) is a volatile liquid, with a faint characteristic, aromatic odour. It is a major petroleum derivative, practically insoluble in water, and is used to synthesize a significant number of high-use organic commodity chemicals, including ethyl benzene, cumene, cyclohexane, aniline, and chlorobenzene. These petrochemicals are used as raw material in the manufacture of plastics, nylons, synthetic fibres, resins, pesticides, pharmaceuticals and detergents. Major anthropogenic sources of benzene include automobile exhaust, point-source emissions from petroleum refineries and processing units, and fugitive emissions from gas stations during refuelling and tank filling procedures. Other sources include tobacco smoke, steel manufacturing, and a wide variety of other industrial activities.

While the average Canadian ambient air levels of benzene in 1998 were 0.3-1.0 $\mu g/m^3$ in rural areas and 1.0 to 3.2 $\mu g/m^3$ in urban areas, there has been a continuing significant trend of decreasing average ambient levels of benzene in Canada since 1990. Benzene is classified as toxic under the Canadian Environmental Protection Act (CEPA, 1993). Benzene is a reportable substance for the National Pollution Release Inventory (NPRI, 2008) of Environment Canada, where steel manufacturing facilities and petrochemical refineries are amongst the largest industrial contributors of benzene releases to the Canadian air shed. Long-term accumulation of benzene in the atmosphere is not expected.

Benzene is rapidly absorbed from both the oral and inhalation routes, while dermal absorption is quantitatively insignificant. After absorption, benzene is distributed throughout the body. Most of the metabolism of benzene takes place in the liver (via CYP2E1), whereas metabolism in other tissues (i.e., bone marrow) has also been identified. The first step in benzene metabolism is the formation of benzene oxide – subsequent metabolism produces many metabolites, some of which are considered ultimately responsible for the carcinogenic activity of benzene. Unmetabolized benzene is also eliminated rapidly.

Numerous findings from *in vivo* and *in vitro* studies indicate that benzene is carcinogenic to both humans and animals. Several types of leukemia have been

observed in the majority of epidemiological studies. However, AML is the subtype of leukemia that has been used by all of the agencies for quantitative dose-response analysis.

Analysis of the mechanism of benzene metabolism is important in understanding the toxicity of benzene. Evidence suggests that the metabolites of benzene, such as benzene oxide, and the products of both the phenol pathway (e.g., catechol, hydroquinone, and p-benzoquinone), and muconaldehyde (e.g., trans, trans-muconic acid), are associated with the toxic effects of benzene. Some of the epidemiological data shows increased incidence and mortality of leukemia at occupational exposure concentrations. The observed carcinogenic outcome from occupational exposures of benzene cannot be solely explained by genotoxic mechanisms.

Recent publications on modes-of-action suggest that both genotoxic and epigenetic mechanisms may be responsible for the toxicological events leading to carcinogenicity at low doses. Weight-of-evidence from recent toxicokinetic and toxicodynamic studies indicate a genotoxic component to benzene carcinogenicity at low doses. Certain observations, on their own, *qualitatively* provide support that either a sublinear, supralinear, or linear response may occur, however the data remains insufficient for *quantitative* dose-response analyses, based on sub- or supralinearity. From a dose-response point of view, low dose linear extrapolation from high doses using linear models is considered to be a common approach for compounds with, in part, a genotoxic mode-of-action. For example, the formation of benzene metabolites follows a linear dose-response relationship. Similarly, the formation of DNA and protein adducts by benzene metabolites follows a linear relationship. Additionally, at environmental benzene exposure doses, the capacity of DNA repair decreases in a linear manner, which results in increased base-pair deletions and dicentric formations. However, some epidemiological studies indicate lower AML mortality than predicted by linear extrapolation from other epidemiological studies.

Considering the overwhelming evidence of the strong carcinogenic potential of benzene, regulatory agencies have established air guidelines towards reducing potential cancer risks. Because the carcinogenic effects of benzene occur at lower levels of exposure than the non-carcinogenic effects, basing guidelines on the mitigation of potential cancer risk also provides the necessary protection against the development of non-carcinogenic effects.

There is currently no Ambient Air Quality Criteria (AAQC) in Ontario for benzene. In revising the air quality standards for Ontario, the Ministry of the Environment is considering risk assessments and standards and guidelines used by environmental agencies world-wide. This document reviews the air quality guidelines and standards developed, recommended, or adopted by the States of California, Louisiana, Massachusetts, Michigan, New Jersey, New York, North Carolina, and Texas, and the countries of Canada (Health Canada), France, UK, The Netherlands (RIVM), Sweden, and the U.S. (US EPA), the European Union (EU), and the World Health Organization (WHO).

Many regulatory agencies have used the Pliofilm cohort as the best published data set to quantitatively evaluate human cancer risks from exposure to benzene. Agencies that

use this study, at least in part, to develop inhalation unit risks include Health Canada, US EPA, RIVM, the EU, and the WHO. Further meta-analyses of data from petrochemical industries used by the EU and RIVM point to some inconsistencies in observed mortality rates in humans exposed to benzene. As a result, EU and RIVM approaches differ in the methods and assumptions used in the dosimetric extrapolation from occupational exposures to environmental exposures. Thus, there is variability in their respective inhalation unit risks.

The methods of the US EPA and the EU are considered to be the most appropriate approaches, as both employ linear and non-linear models for dosimetric analyses of the Pliofilm data. In both cases, the recommended guideline values were derived from extrapolation of occupational exposure concentrations to ambient air exposures. Both Pliofilm (US EPA and EU) and the petrochemical cohort studies (EU), used by these agencies, recorded mortality due to AML as their endpoint for their dose-response analysis. However, AML death due to environmental benzene exposure is often not observed and thus the cancer risk might have been under estimated. Instead, the method of low dose extrapolation should be guided by the understanding of toxicological events at the cellular level that would eventually lead to leukemia.

There are some key toxicokinetic and toxicodynamic issues specific to benzene which are considered in various studies, when high to low dose extrapolation is performed (i.e., post-2000 studies published subsequent to the US EPA and EU analyses). These recent studies have begun to shed light on the toxicological events which may occur at environmental exposure concentrations – namely, DNA repair kinetics, adduct formation, and the benzene metabolism pathway at low doses. Overall, the molecular toxicological events provide a weight-of-evidence support for a health protective linear extrapolation method for the development of a benzene AAQC.

During the consultation on the SDD for benzene several issues were raised, and the Ministry received comments from stakeholders. The issues raised by the stakeholders included: the applicability of linear extrapolation; inconsistencies in the proposed link between early genotoxic damage, hematological findings and the development of AML; the possibility of a functional threshold; high dose to low dose extrapolation issues, including chemical mixture implications; protection of sensitive subpopulations; and, a number of science-policy issues.

After considering the significant toxicological effects of benzene, especially at low doses, the scientific rationale of other jurisdictions that supports their respective air guidelines, and the stakeholder comments, the Ministry determines that the linear extrapolation of the Pliofilm data (Crump 1994) provides a quality risk analysis on the cancer effects of benzene. Thus the Ministry is proposing the following AAQCs and air quality standards for Benzene:

Proposed Annual Average AAQC for Benzene:

- **An annual average Ambient Air Quality Criterion (AAQC) of 0.45 µg/m^3 (micrograms per cubic metre of air) for Benzene, based on carcinogenicity associated with exposure to Benzene,**

Proposed 24-hour Average AAQC for Benzene:

- **A 24-hour average Ambient Air Quality Criterion (AAQC) of 2.3 µg/m^3, (micrograms per cubic metre of air) for Benzene, based on carcinogenicity associated with exposure to Benzene,**

Proposed 24-hour Average Standard for Benzene:

- **A 24-hour average standard of 2.3 µg/m^3 (micrograms per cubic metre of air) for Benzene, based on carcinogenicity associated with exposure to Benzene, and**

Proposed ½-hour Average Standard for Benzene:

- **A half-hour average standard of 7 µg/m^3 (micrograms per cubic metre of air) for Benzene, based on carcinogenicity associated with exposure to benzene.**

After consultation on these proposed standards, the Ministry's intent is to arrive at a decision regarding the effects-based AAQCs and the corresponding effects-based 24-hour and half hour standards. The AAQCs will be added to Ontario's Ambient Air Quality Criteria and the 24-hour standard and half-hour standard will be incorporated into schedule 3 and Schedule 2 of Regulation 419/05, respectively.

MOE generally proposes a phase-in for new standards or standards that will be more stringent than the current standard or guideline. The phase-in for benzene is set out in O.Reg. 419/05.

Among other things, O.Reg 419/05 sets out the applicability of standards, appropriate averaging times, phase-in periods, types of air dispersion models and when various sectors are to use these models. There are 3 guidelines that support O.Reg 419/05. These guidelines are:

- "Guideline for the Implementation of Air Standards in Ontario" (GIASO);
- "Air Dispersion Modelling Guideline for Ontario" (ADMGO); and
- "Procedure for Preparing an Emission Summary and Dispersion Modelling Report" (ESDM Procedure).

GIASO outlines a risk-based decision making process to set site specific altered air standards to deal with implementation barriers (time, technology and economics) associated with the introduction of new/updated/air standards and new models. The altered standard setting process is set out in Section 32 of O.Reg. 419/05.

For further information on these guidelines and O.Reg. 419/05, please see the Ministry's website at http://www.ene.gov.on.ca/ and follow the links to local air quality.

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1.0 Introduction

Ontario regulates air emissions in order to achieve and maintain air quality which is protective of human health and the environment. The Environmental Protection Act (Section 9) requires that all stationary sources that emit, or have the potential to emit, a contaminant to obtain a Certificate of Approval which outlines the conditions under which the facility can operate.

The Ministry of the Environment uses a combination of regulated point of impingement (POI) standards and guidelines (MOE, 2008) in reviewing Emission Summary and Dispersion Modelling Reports submitted to support a Certificate of Approval application or a Ministry request for a compliance assessment. Ambient Air Quality Criteria form the basis for an air standard or guideline and represent human health or environmental effects based values, normally set at a level not expected to cause adverse effects based on continuous exposure. As such, factors such as technical feasibility and costs are not considered when establishing AAQCs or the equivalent half hour standards which are derived from the AAQCs using a mathematical scaling factor. The risk based process for alternative standards, as set out in section 32 of O. Reg. 419, is the mechanism created to deal with the time, technical and economic issues. The Guideline for the Implementation of Air Standards in Ontario (GIASO) is the supporting document for stakeholders who are interested in more information on alternative standards. For further information on O. Reg. 419 and GIASO, please see the Ministry's website.

Ontario Ministry of the Environment Website: Reg. 419 and GIASO

Air standards referenced in O. Reg. 419 are used for compliance and enforcement. Dispersion modelling, as referenced in the regulation, is used to relate emission rates from a source to resulting concentrations of a particular contaminant. Air standards specified under O. Reg. 419 apply to stationary sources only.

In addition to air standards established under O. Reg. 419, the Ministry also has a large number of guidelines (including AAQCs). Similar to standards, guidelines are used by the Ministry to assess general air quality and the potential for causing adverse effect (MOE, 2008). Like the air standards specified in O. Reg. 419, guidelines (and now AAQCs) are used in reviewing Emission Summary and Dispersion Modelling reports submitted in support of applications for Certificates of Approval, to approve new and modified emission sources or other requirements. Once incorporated into a legal instrument such as a Certificate of Approval, guidelines can become legally binding.

The Ontario Ministry of the Environment continues to develop and/or update air standards for priority toxic contaminants. The Ministry's Standards Plan, which was released in October 1996 and revised in November 1999 (MOE, 1999; MOEE, 1996), identified candidate substances for which current air standards will be reviewed.

Benzene has been targeted for air standards development based on the pattern of use in Ontario and the identification of significant toxicological information for this compound. This rationale Document provides a review of scientific information relevant

to setting an Ambient Air Quality Criteria (AAQC) level and Point of Impingement (POI) standard for benzene.

In the 1999 Standards Plan, MOE made a commitment to consider time, technical, and economic issues for air standards and develop a risk management framework to address implementation issues. The risk-based framework has been developed and is part of O.Reg. 419/05. The alternative standards setting process is a risk-based process that considers time, technical and economic issues on a site specific basis. For further information on Regulation 419/05 and the process for requesting an alternative site specific air standard, please see the Ministry's website and follow the links to local air quality

Overview of the Phases in the Ministry's Air Standards Setting Process

The MOE's 1999 Standards Plan outlined a multi-step process for developing air quality standards (MOE, 1999). In response to stakeholder request, and in consultation with stakeholders, the ministry has revised the way it consults on air standards from that described in the Standards Plan.

Previously, as an initial step, risk assessment information relevant to establishing a standard for a particular compound, was documented and posted on the Environmental Registry for stakeholder review in the form of an "Information Draft". This provided stakeholders with the opportunity to review the information and provide any additional information that should be considered by the Ministry in setting an air quality standard for a particular compound. The Ministry then considered this information in developing the "rationale Document" for the compound(s) in which document the Ministry proposed a numerical value(s) for each substance(s).

This has now been changed to partially combine the two steps into a Science Discussion Document. The Science Discussion Document contains more scientific analysis than the former Information Draft; however no regulatory limit is proposed. Science Discussion documents are e-mailed to stakeholders interested in that substance, and followed by a science discussion meeting to enable stakeholders to discuss the science with the ministry and each other. Written comments can also be submitted commenting on the Science Discussion Document. This is referred to as the '**pre-consultation'** step (or phase).

After giving consideration to outstanding issues which may arise from the pre-consultation phase, a rationale Document is prepared that undergoes formal public consultation by posting on the EBR. This is referred to as the '**rationale document'** phase.

The posting of the rationale Document provides an opportunity for comments from stakeholders regarding the proposed ambient air quality criteria and Point-of-Impingement standard. The goal of the rationale document phase is to arrive at a decision regarding the effects-based AAQCs and the corresponding effects-based POI standard for benzene. The final decision is also posted on the EBR as a Decision Document. If the proposed effects-based standards cannot be met immediately, this will be dealt with on a case-by-case basis under Regulation 419 and its associated guidelines.

In the 1999 Standards Plan, MOE made a commitment to consider time, technical, and economic issues for air standards and develop a risk management framework to address implementation issues. The risk-based framework has been developed and is part of O.Reg. 419. The alternative standards setting process is a risk-based process that considers time, technical and economic issues on a site specific basis. For further information on Regulation 419 and the process for requesting an alternative site specific air standard, please see the Ministry's website and follow the links to local air quality.

2.0 General Information

Benzene is an aromatic volatile organic compound previously used as a multi-purpose industrial organic solvent. Upon its recognition as a known carcinogen, its use as a solvent has become significantly diminished. Currently, benzene is primarily used as a precursor in organic chemical syntheses. Based on epidemiological studies, and toxicological studies in animals, the development of leukemia appears to be the target for adverse effects following chronic exposure to benzene by inhalation, where the key criterion for assessing the risk of inhalation exposure to benzene is its carcinogenic potential (see Sections 3, 4 and 5). As such, this document will be focussed primarily on the inhalation route of exposure, but will highlight data derived from other routes of exposure, where inhalation data are lacking, or other route-specific points are to be made.

2.1 Physical and Chemical Properties

Benzene (C_6H_6) (CAS# 71-43-2) is a volatile liquid (ATSDR, 2007). It has a faint characteristic, aromatic odour. A number of physical-chemical properties of benzene are listed below:

Molecular formula: C_6H_6

Molecular weight: 78.11 g/mol

Boiling point: 80.1^{o}C

Melting point: 5.5^{o}C

Henry's Law Constant: 5.5 x 10^{-3} atm-m^3/mol

Vapour pressure & reference temperature: 75 mm Hg at 20^{o}C

Water solubility & reference temperature: 0.188% at 25^{o}C

Log K_{ow}: 2.13 (reference temperature not identified)

Log K_{oc}: 1.8 – 1.9 (reference temperature not identified)

Odour threshold: Water: 2.0 mg/L; Air: 4.9 mg/m^3

Conversion factors:

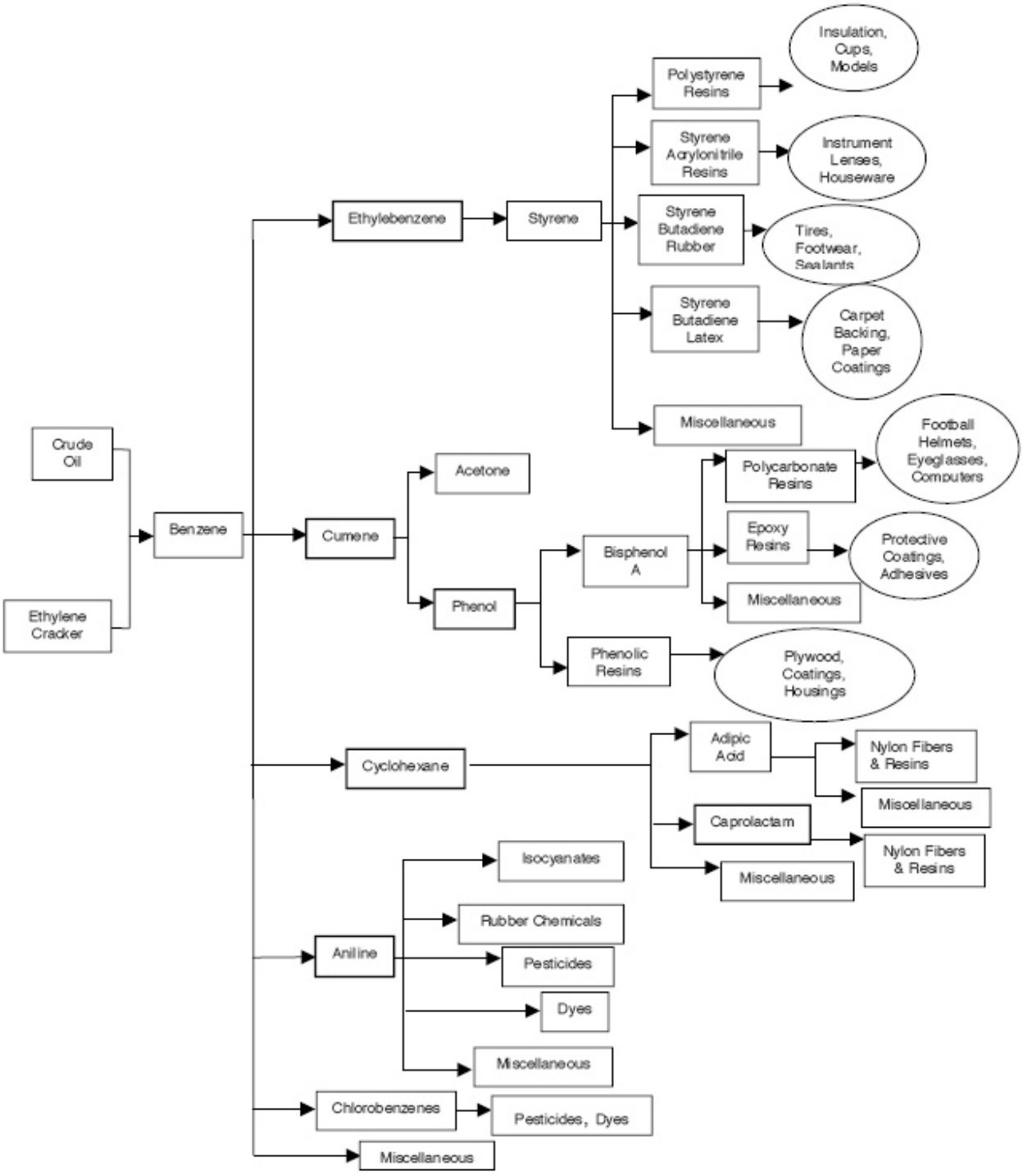
1 ppm = 3.24 mg/m^3 at 20^{o}C and 1 atm

1 mg/m^3 = 0.31 ppm

2.2 Uses and Production of Benzene

Currently, benzene is primarily used as a precursor in organic chemical syntheses. As a major petroleum derivative, benzene is used to synthesize a significant number of high-use organic commodity chemicals, including ethyl benzene, cumene, cyclohexane, aniline, and chlorobenzene. It is these latter petrochemicals which are similarly used to synthesize a number of high-use chemicals, used to manufacture various plastics, nylons, synthetic fibres, resins, pesticides, pharmaceuticals and detergents (HSDB, 2001; USITC, 2003; ATSDR, 2007). A graphical description of the major commodity chemicals and polymers derived from benzene is found in Figure 2.1.

Figure 2.1: Major commodity chemicals and polymers derived from benzene. Source: United States International Trade Commission, 2003



2.3 Sources, Emissions, and Environmental Levels

Benzene is one of the major petroleum hydrocarbons that are released into the environment as a result of a number of anthropogenic activities. It is found in air, water, and soil from both man-made as well as natural sources. Examples of anthropogenic sources include automobile exhaust, point-source emissions from petroleum refineries

and processing units, and fugitive emissions from gas stations during refuelling and tank filling procedures. Other sources include environmental tobacco smoke (passive smoker), steel manufacturing, and a wide variety of other industrial activities.

Proportionally, the major anthropogenic sources of benzene are from combustion of gasoline and diesel fuels, which together account for 76% of the total atmospheric releases (CEPA, 1993). The average benzene concentration in gasoline sold in Ontario is 0.7 to 0.8%, and is representative of the average across Canada. It is worth noting that as recently as 1995, the benzene content in gasoline in Ontario was as high as 1.4 -1.6%. It has also been shown that indoor and outdoor air levels of benzene near sources of benzene emissions are higher than the ambient air concentration (WHO, 2000).

Compared to anthropogenic sources, the contribution of natural sources of benzene to the atmosphere is generally not significant. As mentioned above, benzene is derived from crude oil. It is also released during forest fires (Graedel, 1978; Brief *et al*., 1980), as well as from volcanoes (ATSDR, 2007). While the average Canadian ambient levels of benzene in 1998 were 0.3-1.0 µg/m^3 in rural areas and 1.0 to 3.2 µg/m^3 in urban areas (CCME, 2001), there has been a continuing trend of decreasing average ambient levels of benzene in Canada since 1990 – a decrease of about 4-fold (Figure 2.2).

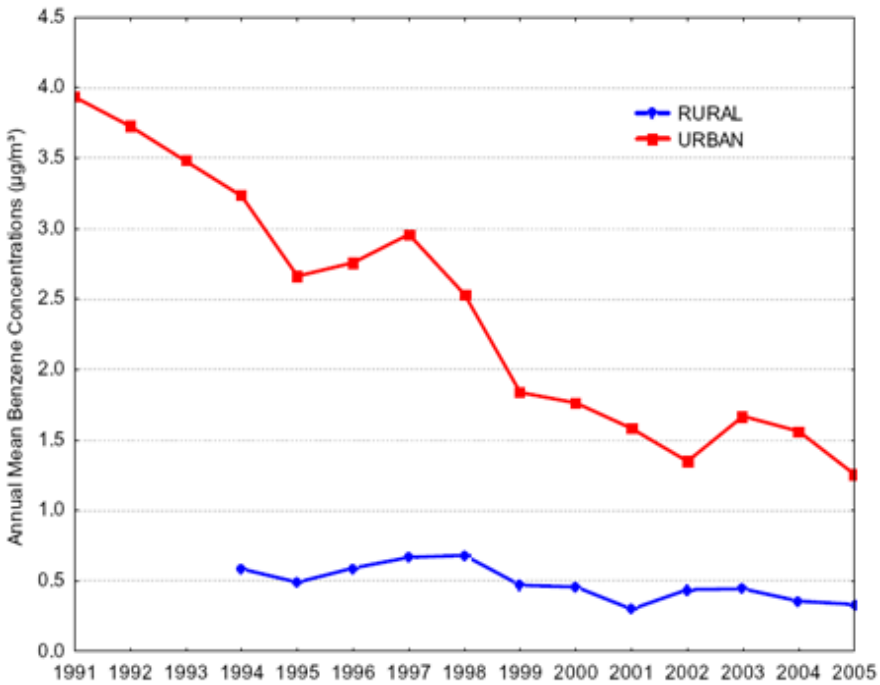
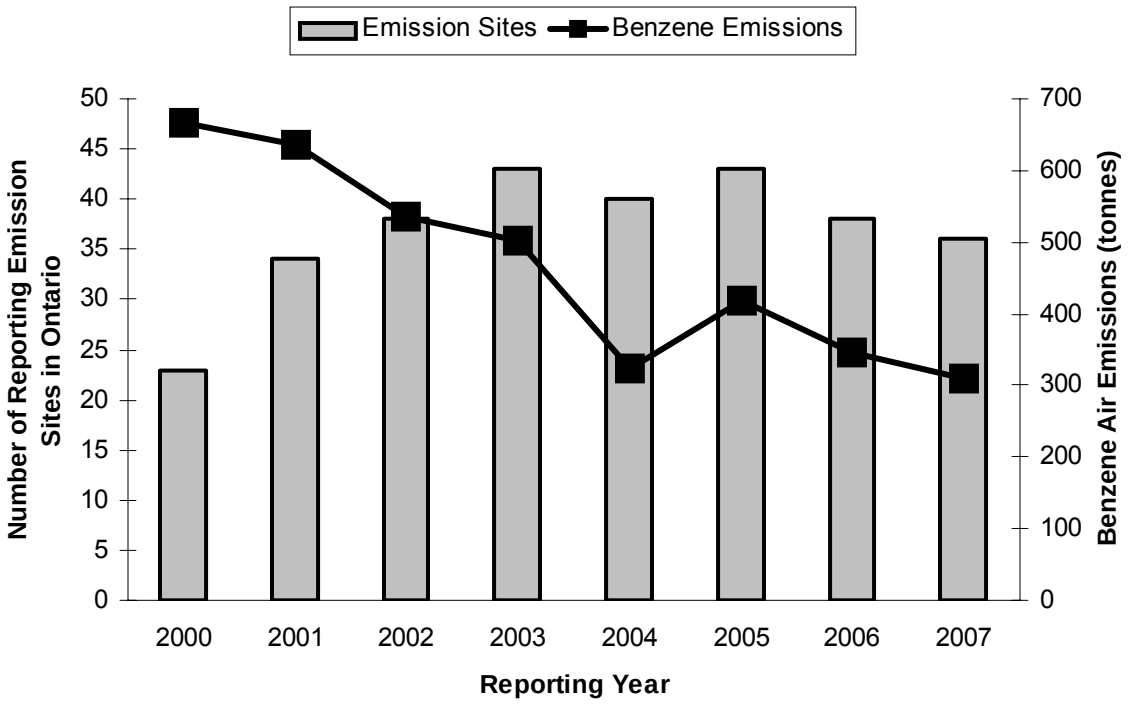


Figure 2.2: Time course concentration profile of benzene in Canadian rural and urban environments. Source: Environment Canada, 2005.

Benzene is classified under the Canadian Environmental Protection Act (CEPA) as toxic. Benzene is a reportable substance for the National Pollution Release Inventory (NPRI) of Environment Canada (NPRI, 2008), where steel manufacturing facilities and petrochemical refineries are amongst the largest industrial contributors of benzene releases to the Canadian air shed.

When looking at the reported NPRI data for Ontario emissions, while the number of reporting benzene emitting facilities has increased between 2000 and 2007, the overall amounts of benzene emitted has decreased steadily in Ontario (Figure 2-3). It is worth noting that three steel manufacturing sites (of the 38 reporting emitters) accounted for over 64% of the reported 347 tonnes of benzene air emissions in Ontario, based on data from the 2006 reporting year.

Figure 2.3: Benzene emission sites and emission amounts in Ontario, for the reporting years 2000 to 2007. Data derived from National Pollution Release Inventory website (NPRI, 2008). Note: the data for year 2007 has not been reviewed by NPRI (as of October 30, 2008).



It should be noted that the Canada-wide benzene standard of the Canadian Council of Ministers of the Environment (CCME) is structured as a two-phased approach for the reduction of benzene concentration in the atmosphere, to be accomplished through emission reductions (CCME, 2001). The first phase consists of a 30% reduction strategy, whereas the second phase targets a 6 kilotonne national emissions reduction strategy by the year 2010.

2.4 Environmental Fate

Released benzene is not stable, and undergoes various degradation processes in the air. Photo-oxidation is the major degradation pathway for benzene in air (Japar *et al* 1991). Benzene is oxidized in reactions with hydroxyl radicals and, to a lesser extent, tropospheric ozone and nitrate radical (NO_3) (CEPA, 1993). In an atmosphere with a hydroxyl radical concentration of 1.1 x 10^6 molecules/cm^3 it is estimated that the residence time of benzene would be 8 days (ATSDR, 2007). Major products of photo-oxidation include: phenol, nitrophenol, nitrobenzene, glyoxal, butanedial, formaldehyde, carbon dioxide, and carbon monoxide (Nojima *et al*., 1975; Finlayson-Pitts and Pitts, 1986). Since the atmospheric half-life of benzene is relatively short, long-range transport of benzene is unlikely.

Volatilization and biodegradation are the major processes involved in the removal of benzene from water. The primary mechanisms responsible for loss of benzene from soil are volatilization to the atmosphere and runoff to surface water. Between the combinations of direct sunlight in oxygen saturated deionized water, a half-life of 16.9 days was observed (ATSDR, 2007). Half-lives of benzene in river water, 16 days, and groundwater, 28 days, have also been reported (ATSDR, 2007). The half-life in water approximately 1 meter deep is estimated to be 4.8 hours (ATSDR, 2007). Finally, biodegradation also accounts for a small proportion of loss (CEPA, 1993).

Taken together, long-term accumulation of benzene in the atmosphere is not expected.

3.0 Toxicology of Benzene

The toxicity of benzene is well documented in the scientific literature. Although the following toxicological review of benzene is focused on the inhalation route of exposure, data from other routes have been included where data on inhalation exposures may be limited, or if novel insights from other routes are to be garnered. More specifically, the following review will focus on the toxicological studies considered relevant by other regulatory agencies in the development of their air quality criteria document for benzene, and therefore, by no means should be considered a definitive review of benzene toxicity or epidemiological studies.

In general, benzene inhalation exposure at different concentrations and different durations may lead to adverse effects as diverse as reversible neurological symptoms such as dizziness and headache (acute exposure), to hematological effects such as anemia and leukemia (Ayres *et al*., 1994). Benzene is classified as a known genotoxic carcinogen, and is associated with severe health concerns, including leukemia (Duarte-Davidson *et al*., 2001). In developing an air standard, the focus is on the most sensitive effect on the general population, as a result of chronic continuous low exposure to benzene.

It should be noted that routes of exposure other than inhalation are cited in this document, in order to rule out the possibility that there is no evidence of increased toxicity by contributed by other routes of exposure than inhalation, as benzene is a systemic toxic.

3.1 Toxicokinetics

Prior to an overview of the adverse human health effects of benzene exposure, it is worth discussing the toxicokinetics of benzene. Toxicokinetic evaluation of benzene through different routes of entry has been carried out extensively in several species of experimental animals and in humans (Rusch *et al*., 1977; Snyder *et al*., 1980; Sabourin *et al*., 1988a; Sabourin *et al*., 1988b; Medinsky *et al*., 1989; Sabourin *et al*., 1989; Seaton *et al*., 1994; Ong *et al*., 1995; Witz *et al*., 1996; Bruckner and Warren, 2001; Turteltaub and Mani, 2003; Lin *et al*., 2006; Snyder, 2007). Briefly, benzene is rapidly absorbed from both the oral and inhalation routes. While dermal absorption is also rapid, quantitatively dermal absorption is very low due to rapid evaporation from skin. After absorption, benzene is distributed throughout the body and accumulates in fatty tissues. Most of the metabolism of benzene takes place in the liver and to a lesser extent, in the bone marrow, which is considered to be critical in the development of leukemia.

As will be discussed below, the characteristic hematotoxic and carcinogenic effects of benzene are attributed to the metabolites of benzene. This is an important fact with regards to regulatory toxicology, as at low environmental exposure concentrations benzene is rapidly metabolized and excreted primarily as conjugated metabolites. However, at higher doses (i.e., at some doses observed in some epidemiological studies), these metabolic pathways become saturated, and excretion of non-

metabolized benzene is observed as a significant process. This concept will be further discussed in Section 3.8, entitled Low dose Considerations.

While the pathways of benzene metabolism appear to be qualitatively similar across species, significant quantitative differences in metabolism across species are observed. Despite the breadth and depth of benzene research, there still lacks a universally accepted model for human metabolism of benzene (US EPA, 2000; ATSDR, 2007). In fact, despite the development of several physiologically based toxicokinetic (PBTK) models – discussed below – extrapolation of results from laboratory animal studies to humans has proven to be fraught with uncertainties. However, tracking the parent compound, and time profiling its internal dosimetry, may be useful in understanding the toxic mechanisms of benzene. As such, a detailed discussion of each toxicokinetic stage of benzene is presented in the following sub-sections.

3.1.1 Absorption

There are numerous animal studies which describe the absorption profile of benzene at various concentrations. Schrenk *et al.* (1941) described a linear relationship between benzene concentration (639–4153 mg/m^3) and the equilibrium concentration of benzene in the blood of dogs. A steady-state blood level was attained within 30 minutes at these exposure concentrations, revealing the fact that the benzene half-life is short. As well, this study demonstrated that there is quick metabolic saturation at these high levels of exposure, thus leading to steady state build up. Indeed, exposure concentration appears to affect the lung retention of inhaled benzene tagged radioactivity (Sabourin *et al*., 1987). Here, the percentage of lung retention of benzene by rats and mice during a 6 hour exposure decreased (33 ± 6% to 15 ± 9% for rats, and 50 ± 1% to 10 ± 2% for mice), as exposure concentration increased (26 to 2600 mg/m^3). This study also showed species variability in the uptake and retention of inhaled benzene. At all exposure concentrations, absorption was higher in mice. Specifically, at exposure concentrations below 350 mg/m^3, mice retained approximately 50% more radioactivity per kilogram body weight than did rats, but there was no significant difference at the highest (2500 mg/m^3) concentration.

Additionally, there is a significant experimental database on the respiratory absorption of benzene in humans (Srbova *et al*., 1950; Nomiyama and Nomiyama, 1974; Pekari *et al*., 1992; Yu and Weisel, 1996), with rather consistent results. For example, Srbova *et al*. (1950) examined absorption of benzene in 23 human subjects exposed to a range of concentrations, from 150 to 320 mg/m^3, for two to three hours. Absorption was greatest in the first five minutes of exposure (70–80%) but declined rapidly over the next 15 minutes, where it varied after 1 hour (20–60%) and 2 hours (20–50%) of exposure. Nomiyama and Nomiyama (1974) determined both retention and uptake of benzene in 3 female and 3 male subjects, 18–25 years of age, exposed to 166–198 mg/m^3 for 4 hours. Retention declined from approximately 50% in the first hour, and stabilized at 30% after 3 hours. Respiratory uptake averaged 47%, with excretion of 17%. Pekari *et al*. (1992) studied the respiratory absorption of benzene in 3 males exposed to 5 and 39 mg/ m^3 benzene for 4 hours, and found that the absorption was about 50% at both concentrations. Yu and Weisel (1996) measured the uptake of benzene (102 to 220 mg/m^3) from side stream tobacco smoke by 3 female subjects. Absorption in eight

separate experimental runs averaged 64%, with a range of 48–73%. The exposure periods were either 30 or 120 minutes, however, unlike the other experiments described, no significant decrease in absorption with the longer exposure period was observed.

The observed decline in absorption with increasing exposure time is apparently due to respiratory excretion of unmetabolized benzene, thus leading to a lack of transient steady state exposure concentration in the lung. Thus, with the exception of the study by Pekari *et al*. (1992), benzene metabolism may have been saturated in the human respiratory absorption studies, and would be expected to lead to greater respiratory excretion of unmetabolized benzene. Therefore, above 32 mg/m^3 (or 10 ppm), the rate of formation of the metabolites of benzene is limited and the effects due to the parent compound, benzene, may play a predominant role in adverse effects.

Finally, it is worth noting that although only limited data are available on absorption of benzene in humans by the oral route, accidental or intentional poisoning case studies indicate that benzene is readily absorbed by this route (Thienes and Haley, 1972). Conversely, dermal absorption is considered to be minimal and will not be further involved in this discussion.

3.1.2 Distribution

There is extensive information on benzene distribution after oral, inhalation, and dermal routes of exposures in animals, and has been extensively reviewed by ATSDR, 2007. Benzene distribution after inhalation exposure is discussed as below.

Results from animal studies indicate that absorbed benzene after inhalation exposure is distributed throughout several body compartments. In general, the relative tissue uptake appears to be dependent on the blood perfusion rate within the tissue, as well as the partitioning effect between blood and the specific tissue. For example, fat tissue is expected to accumulate more because of benzene's high lipid solubility. Steady-state benzene concentrations in rats exposed via inhalation to 1600 mg/m^3 for 6 hours were 1.2 mg/dL in blood; 3.8 mg/dL in bone marrow; and 16.4 mg/dL in fat (Rickert *et al*., 1979). Benzene was also found in the kidney, lung, liver, brain, and spleen. Levels of the benzene metabolites phenol, catechol, and hydroquinone were higher in the bone marrow than in blood, with phenol being eliminated more rapidly than catechol or hydroquinone after exposure. Pregnant mice exposed to a benzene concentration of 6400 mg/m^3 for 10 minutes had benzene and its metabolites detected in lipid-rich tissues such as brain and fat, as well as in highly perfused tissues such as liver and kidney (Ghantous and Danielsson, 1986). Benzene was also found in the placenta and fetus immediately following exposure. Benzene was rapidly distributed throughout the bodies of dogs exposed via inhalation to concentrations of 2556 mg/m^3 for up to 8 hours per day, for 8 to 22 days (Schrenk *et al*., 1941). Fat, bone marrow, and urine contained about 20-fold the concentration of benzene in blood; benzene levels in muscles and organs were 1- to 3-fold that of blood, and erythrocytes contained about twice the amount of benzene found in plasma.

Studies in pregnant mice demonstrated that after inhalation exposure, ^{14}C-benzene crossed the placenta. Volatile radioactivity (i.e., unmetabolized benzene) was observed

in the placenta and foetus immediately after and up to 1 hour after exposure (Ghantous and Danielsson, 1986). Non-volatile metabolites were also detected in the foetus, but at lower levels than in maternal tissues. The labelled signal peaked in fetal tissues 30–60 minutes after inhalation, similar to the peak observed for maternal tissues. No firmly tissue-bound metabolites of benzene were detected in the fetal tissues in late gestation, indicating that the mouse foetus did not have the ability to form the reactive metabolites, and thus the carcinogenic effect is thought to be less active in the fetus.

In humans, Sato *et al*. (1975) compared elimination kinetics of benzene in men and women of similar ages. Exposure was for 2 hours at 80 mg/m^3. The level of benzene in the blood and the end-tidal air was different for males and females, with the shape of the decay curve significantly steeper in the males. The authors attributed these results to the higher fat content of females.

3.1.3 Metabolism

This section provides an overview of benzene metabolism. Specifically, the relevance of toxic effects of the metabolites of benzene at environmental exposure levels will be the focus. Key to this discussion is an appreciation of how the metabolism of benzene to its metabolites is thought to be critical to its toxicology, and to the progression of leukemia, as presented in Figure 3-1.

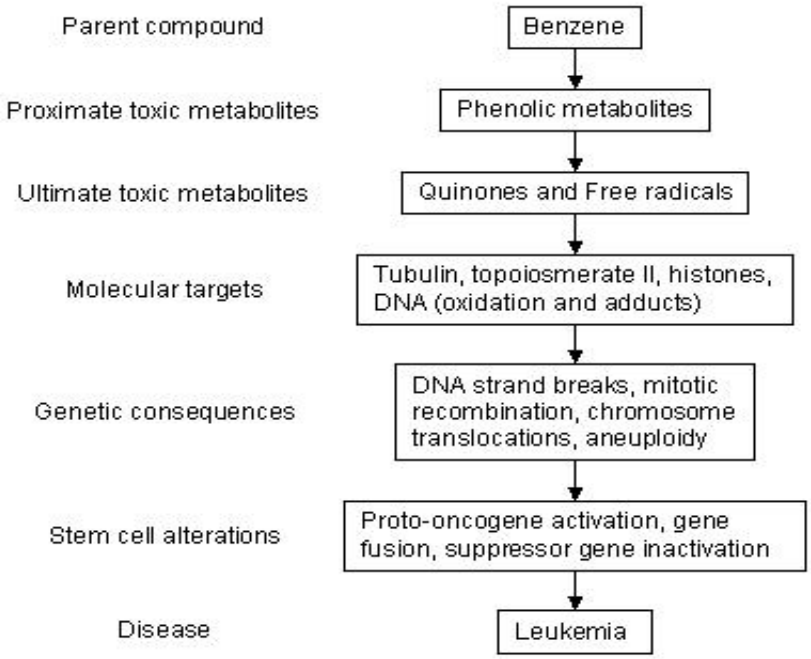


Figure 3.1: Schematics of the mechanistic hypothesis of benzene leukomogenesis. Source: modified from US EPA (1998)

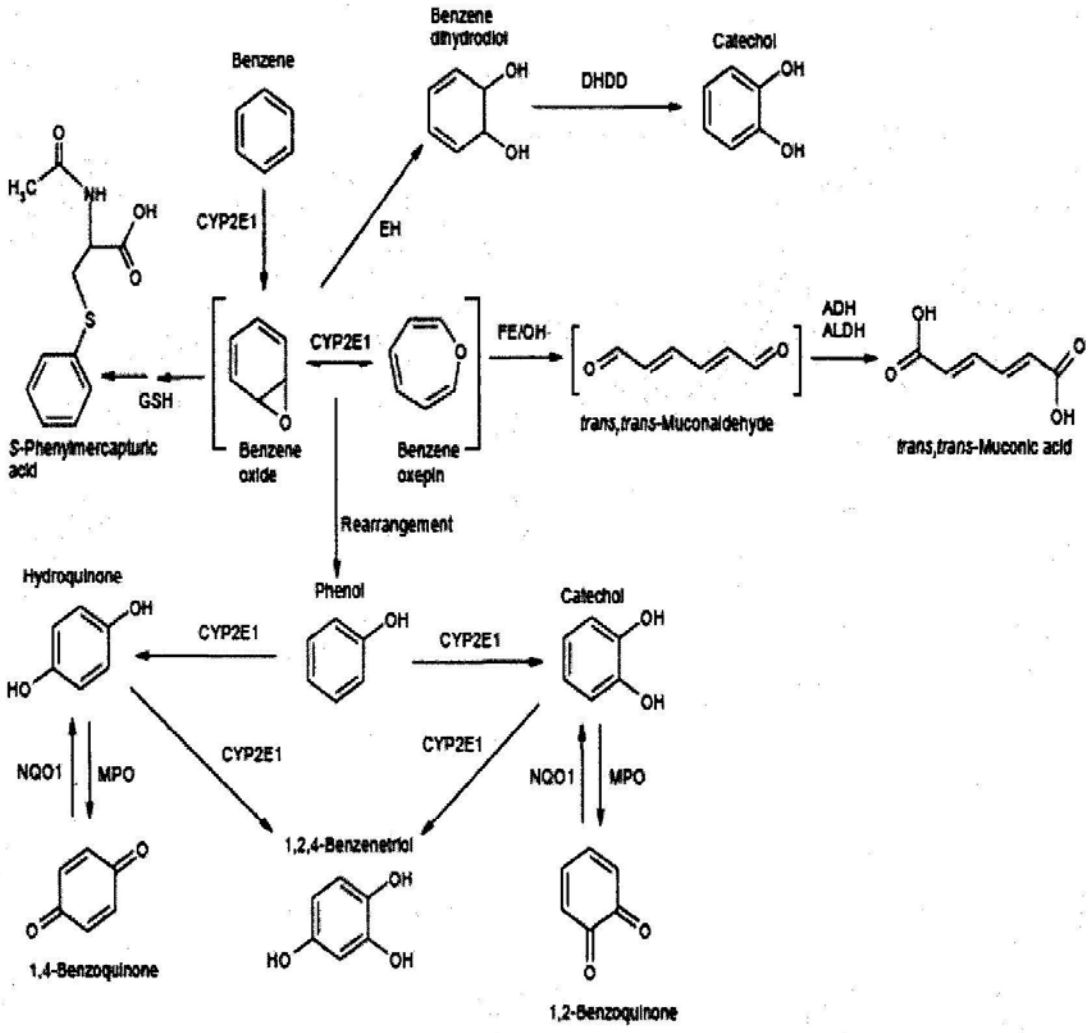
The production of the initial benzene metabolites occurs primarily in the liver, yet the effects of benzene toxicity are primarily expressed as hematotoxicity and myelotoxicity in the bone marrow (Snyder and Hedli, 1996). Overall, the selective toxicity of any individual benzene metabolite to blood and blood forming cells (i.e., bone marrow) has been difficult to explain, so these metabolites are viewed as proximate. Secondary activation to toxic quinones and free radicals, or ultimate toxic metabolites, are postulated to take place by peroxidase enzymes in the bone marrow – a hypothesis which has been supported by the fact that target organs in rodents are rich in peroxidase and sulphatase enzymes (US EPA, 1998). Molecular targets for the action of these metabolites, whether acting alone or in concert, include tubulin, histone proteins, topoisomerase II and other DNA associated proteins. Damage of these proteins may potentially cause DNA strand breakage, mitotic recombination, chromosomal translocations, and malsegregation of chromosomes to produce aneuploidy. If these effects occur in stem or early progenitor cells, it is believed that a leukemic clone with advantage to grow could arise as a result of protooncogene activation, gene fusion and suppressor gene inactivation. Epigenetic effects of benzene metabolites on the bone marrow stroma, and perhaps the stem cells themselves, could then foster development and survival of a leukemic clone (US EPA, 1998). In later sections, further evidence of the mode of action of benzene, especially in benzene's ability to affect chromosomal organization at environmental exposure concentrations, will be discussed. In this section, however, we shall highlight the critical importance of benzene metabolism in the toxicological pathway.

Metabolic Pathways

Despite extensive research, the metabolism of benzene is still not thoroughly understood. However, it is generally accepted that benzene itself is not directly responsible for causing carcinogenic effects. Similarly, the metabolic product or products responsible for carcinogenic effects of benzene exposure have not been clearly defined. The available evidence suggests that several metabolites, as well as interactions between these metabolites, may underlie the carcinogenic effects of benzene. A complete review of the metabolism of benzene is beyond the scope of this review, and the reader is referred to several in-depth reviews for more complete discussion (Snyder *et al*., 1993; Ross *et al*., 1994; Snyder and Hedli, 1996; Witz *et al*., 1996; US EPA, 1998; ATSDR, 2007). However, a critical point to state is that all routes of absorption share the following metabolic pathways.

The complexities of the metabolic pathways of benzene are evident in Figure 3-2. Here, the first step in benzene metabolism is the formation of the epoxide, benzene oxide, which is catalyzed by cytochrome P450 2E1 (CYP2E1). After formation of the benzene oxide, the metabolic pathway branches into several alternative metabolic pathways (Jerina *et al*., 1968; Lovern *et al*., 1997). Benzene oxide rearranges nonenzymatically to form phenol, the major product of initial benzene metabolism. Alternatively, benzene oxide may react with glutathione (GSH) to form phenylmercapturic acid, undergo enzymatic conversion by epoxide hydrolase to benzene dihydrodiol with subsequent formation of catechol, or undergo an iron-catalyzed, ring-opening reaction to form trans, trans-muconaldehyde (MUC) with subsequent metabolism to trans, trans-muconic acid

(MA). Phenol is further oxidized by CYP2E1 to the product hydroquinone (Nebert *et al*., 2002). As well, further oxidation of hydroquinone to p-benzoquinone may be catalyzed by the enzyme myeloperoxidase (MPO) (Smith *et al*., 1989). In general, all of the phenolic products may be conjugated with sulfate or glucuronic acid, where the conjugates of phenol and hydroquinones are the major benzene metabolites excreted in urine (Sabourin *et al*., 1989; Wells and Nerland, 1991).



Adapted from Nebert et al. 2002; Ross 2000

ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase; CYP2E1 = cytochrome P-450 2E1; DHDD = dihydrodiol dehydrogenase; EH = epoxide hydrolase; GSH = glutathione; MPO = myeloperoxidase; NQ01 = NAD(P)H:quinone oxidoreductase

Figure 3.2: The metabolic pathways of benzene. Source: TCEQ 2007; adopted from Ross, 2000; Nebert et al. 2002

There have been a number of publications that have examined the kinetics of this metabolic pathway in different species, and at different doses. For example, Mathews *et al*. (1998) studied the metabolism of ^{14}C-benzene after oral dosage in male F344 rats (0.02, 0.1, 0.5, 10, 100 mg/kg-bw), male B6C3F1 mice (0.1, 100 mg/kg-bw), and male hamsters (0.02, 0.1, 100 mg/kg-bw). In rats, at lower doses (0.02, 0.1, and 0.5 mg/kg-bw), greater than 95% of the dose was recovered in the urine within 48 hours, and a small percentage (about 3%) was recovered in the breath. At higher doses, (10 and 100 mg/kg-bw), the percentage eliminated in the breath increased to about 9 and 50%, respectively. Excretion in the feces was a minor route at all doses. A similar pattern of disposition of the radiolabel dose was also observed in mice and hamsters. The authors also examined the profile of urinary metabolites formed. Percentages of prephenylmercapturic acid and phenylmercapturic acid, indicators of benzene oxide production, were relatively constant across all doses for rats (~13%), mice (~5%), and hamsters (~7%). However, the percentage of hydroquinone and related conjugates ranged from about 3% at the highest dose to as much as 7% at the lowest doses. A higher percentage of hydroquinone metabolite was observed in mice (~30%) and in hamsters (~30%), but it did not appear to be dose-dependent.

Requirement for CYP2E1

In general, the oxidation of benzene by CYP2E1 is a complex phenomenon and has been demonstrated to be required for the expression of hematotoxicity and genotoxicity, and for the ultimate development of leukemia. In 1996, Smith proposed a hypothesis based on the indication that benzene produces chromosomal damage both *in vivo* and *in vitro* (Wolman, 1977; Dean 1985; Sasiadek and Jagielski 1990; Yager *et al*. 1990), and concluded that the genotoxic effects produced by benzene are not simple point mutations, but rather recombination and chromosomal aberrations. Benzene exposure has been found to induce CYP2E1 activity, thereby potentially increasing the rate of toxic metabolite formation. Pretreatment of mice, rats, and rabbits subcutaneously with benzene increased benzene metabolism *in vivo* without increasing total cytochrome P450 concentrations (Arinc *et al*., 1991; Gonasun *et al*., 1973; Saito *et al*., 1973). In contrast, Sabourin *et al.* (1990) found no significant effect on the metabolism of benzene when F344 rats and B6C3F1 mice were pretreated by inhalation exposure to 600 ppm (~1950 mg/m^3) of benzene. The rate of benzene metabolism can be altered by pretreatment with various compounds. CYP2E1 also metabolizes alcohol and aniline, and CYP2E1 can be induced by these substrates (Parke, 1989; Chepiga *et al*., 1991; Snyder *et al*., 1993). Phenol, hydroquinone, benzoquinone, and catechol have also been shown to induce CYP450 in human hematopoietic stem cells (Henschler and Glatt, 1995). Conversely, Daiker *et al*. (2000) found that repeated oral benzene exposure of female B6C3F1 mice for 3 weeks at 50 mg/kg/day decreased CYP2E1 activity by 34% and activated the detoxification enzyme GSH transferase by 30% without affecting aldehyde dehydrogenase, another detoxifying enzyme. The authors suggested that these changes in enzyme activity may serve a protective role against repeated benzene exposure. Despite this latter finding, evidence remains suggesting that pre-exposure to chemicals such as benzene, toluene, ethyl benzene, xylenes etc. have the ability to stimulate the activity of CYP2E1 prior to subsequent benzene

exposures. This, in turn, may increase the rate of benzene metabolism, through increased enzyme activity, thus leading to a rise in production of toxic benzene metabolites.

Probably the most convincing evidence associating CYP2E1 activity with benzene toxicity was provided by experiments involving mice which were designed to lack the CYP2E1 gene – that is, transgenic knockout mice that lack hepatic CYP2E1 activity (Valentine *et al*., 1996). Whereas, in wild-type mice, benzene exposure at 640 mg/m^3 for 6 hours per day for 5 days resulted in severe genotoxicity and cytotoxicity, no such toxicity was observed in CYP2E1 knockout mice.

Bernauer *et al*. (2000) investigated the role of CYP2E1 expression in bone marrow and its intra- and interspecies variability in rats, rabbits, and humans, because bone marrow is a target organ for several chemicals, including benzene. Briefly, CYP2E1 was detected in the bone marrow of all species investigated, thus supporting the hypothesis of CYP2E1-dependent local metabolism of several chemicals as a factor possibly contributing to the myelotoxicity and hematotoxicity of these chemicals. While intraspecies/intrastrain variability of CYP2E1 activity in rodents is small, CYP2E1 activity was quite different between rodents and non-rodent species indicating considerable interspecies variability. Immunoinhibition studies in rats and rabbit hepatic microsomes also have implicated CYP2E1 as the major oxidative isoenzyme involved in benzene metabolism (Johansson and Ingelman-Sundberg, 1988; Koop and Laethem, 1992). However other iso-enzymes like CYP2B1, CYP2F2 may also be involved in initial benzene metabolism (Powley and Carlson, 2000; Powley and Carlson, 2001; Sheets and Carlson, 2004; Sheets *et al*., 2004). This may further affect or complicate either the rate of metabolism of benzene and/or the amount of metabolites formed. Sammett *et al*. (1979) showed that partial hepatectomy of rats diminished both the rate of metabolism of benzene and its toxicity, suggesting that a metabolite and/or metabolites formed in the liver are necessary for toxicity.

While the primary oxidation of benzene by CYP2E1 occurs in the liver, further metabolism to the final toxic compound occurs in the target tissues (i.e., via CYP2E1 and/or peroxidase enzymes in the bone marrow). As will be discussed, these and other data demonstrate a presence of CYP2E1 in the bone marrow of a number of species, thus supporting the hypothesis that local metabolism of several metabolites, or possibly benzene itself, contributes to the development of myelotoxicity and hematotoxicity.

Toxicity of Benzene Metabolites

While the requirement for CYP2E1 activity for the metabolism of benzene has been established, the identity of the benzene metabolites responsible for toxicity remains elusive (US EPA, 1998). Those benzene metabolites which are proposed to be key contributors of the toxic effects of benzene exposure – namely, benzene oxide, the products of the phenol pathway (catechol, hydroquinone, and p-benzoquinone), and muconaldehyde (MUC) – are highlighted below.

Benzene Oxide: Lovern *et al*. (1997) showed that benzene oxide constituted 7% of the benzene metabolites after 18 minutes of incubation with liver microsomes. Lindstrom *et al*. (1997) demonstrated the presence of benzene oxide in the blood and estimated its

half-life to be about 8 minutes. Using a PBTK model, Lindstrom *et al*. (1997) predicted that the dose to the body from benzene oxide would be approximately 22-fold greater than that from 1,4-benzoquinone, at any particular benzene exposure concentration. Benzene oxide-protein adducts have been found in the blood and bone marrow of mice exposed to benzene (McDonald *et al*. 1994), and benzene oxide hemoglobin and albumin adducts have been detected in the blood of workers exposed to benzene (Yeowell-O'Connell *et al*. 1998; Rappaport *et al*. 2002a; Rappaport *et al*. 2002b). Thus, circulating benzene oxide may be a contributor to the observed DNA and protein adduct formation.

Phenolic Products (catechol, hydroquinone, and p-benzoquinone): Rickert *et al*. (1979) observed that catechol and hydroquinone concentrations persisted in the bone marrow of mice long after blood levels had declined following inhalation exposure. As discussed above, there is evidence to suggest that secondary metabolism in bone marrow is required for the expression of the toxicity of benzene (Schlosser and Kalf, 1989; Subrahmanyam *et al*., 1990; Subrahmanyam *et al*., 1991). While the presence of CYP2E1 has been detected in rabbit bone marrow (Schnier *et al*., 1989), paradoxically CYP2E1 could not be detected in the bone marrow of mice – the species demonstrated to be most sensitive to benzene hematotoxicity (Genter and Reico, 1994).

Phenol, as parent compound, failed to duplicate the toxic effects of benzene (Tunek *et al*., 1981; NCI, 1980). There are several lines of evidence to suggest that a combination of phenol and hydroquinone is needed to cause bone marrow toxicity. In one study (Eastmond *et al*., 2001), intraperitoneal injection of either phenol or hydroquinone alone in mice failed to cause significant bone marrow toxicity – however, co-administration of these metabolites together caused a reduction in bone marrow cellularity with a clear dose-response curve. The presence of phenol apparently stimulated peroxidase-dependent metabolism of hydroquinone to p-benzoquinone. Additionally, increased covalent binding of ^{14}C-hydroquinone was observed in bone marrow when a combination of phenol and hydroquinone was administered to mice (Subrahmanyam *et al*., 1990; Subrahmanyam *et al*., 1991). Legathe *et al*., (1994) measured the area under the blood concentration-time curve (AUC) for phenol and hydroquinone, administered alone or in combination at the same dose levels (Eastmond *et al*. 1987). Co-administration increased the phenol AUC by 1.4-fold and the hydroquinone AUC by 2.6-fold, in comparison to each compound administered alone. The authors suggested that these observations resulted from saturation of the enzymes that form sulfate and glucuronide conjugates of phenolics in the liver. These results suggest that interactions of two or more metabolites of benzene may be necessary to cause the observed bone marrow toxicity.

Muconaldehyde (MUC): There is evidence to suggest that the benzene metabolite muconaldehyde (MUC) is responsible for bone marrow toxicity, which is summarized by Witz *et al*. (1996). MUC has been shown to cause hematotoxicity following short-term exposures. Administration of 2 mg/kg/day MUC to mice for 16 days caused significant decreases in bone marrow cellularity, lymphocytes, red blood cell (RBC) counts, hematocrit, and haemoglobin, and significant increases in white blood cell (WBC) count and spleen weight (Witz *et al*., 1985). Snyder *et al*. (1989) also found that administration of MUC caused bone marrow toxicity in mice, and that co-administration

of MUC and hydroquinone resulted in a dramatic decrease in iron incorporation into red hemoglobin.

Production of MUC is expected as a step in the ring-opening pathway leading to trans, trans-muconic acid (MA) (Figure 3-2). Excretion of MA in the urine was demonstrated in rabbits, mice, rats, cynomolgus monkeys, chimpanzees, and humans (Parke and Williams, 1953a; Parke and Williams, 1953b; Gad-El Karim *et al*., 1985; Sabourin *et al*., 1988a; Sabourin *et al*., 1989: Sabourin *et al*., 1992). Metabolism of MUC to MA has been shown *in vivo* in mice (Witz *et al*., 1990a; Witz *et al*., 1990b). As such, urinary excretion of MA has been used as a sensitive and specific biomarker of benzene exposure in humans. Most significantly, at low doses, urinary MA concentration was found to be linearly correlated with time-weighted average benzene exposure concentrations (Bechtold *et al*., 1991; Bechtold and Henderson, 1993). Furthermore, both hydroquinone and muconic acid have been detected in the urine of benzene-exposed workers (Rothman *et al*., 1998).

Although MUC formation has not been demonstrated in animals *in vivo*, formation of MUC from benzene has also been demonstrated in a mouse hepatic microsomal system (Latriano *et al*., 1986; Zhang *et al*., 1995a). Additionally, using isolated rat livers perfused with 0.7 mM benzene solutions through the portal vein, Grotz *et al*. (1994) demonstrated that while the complete pathway for formation of MA from benzene was active in the liver, MUC was not detected in the perfusate collected via the hepatic vein. When MUC was added to the perfusion solution, it was rapidly and efficiently metabolized to MA, and only traces of MUC were detected in the perfusate collected after a single pass through the isolated rat livers. Thus, it seems unlikely that sufficient quantities of MUC could reach the target tissues by circulation in the blood. The acid-alcohol 6-hydroxy-trans,trans-2,4-hexadienoic acid (COOH-M-OH) and MA are the major MUC metabolites detected in the rat liver perfusate (Grotz *et al*., 1994). COOH-M-OH has been demonstrated to react with GSH, to be cytotoxic to isolated rat hepatocytes, and to be hematotoxic in mice (Goon *et al*., 1993; Zhang *et al*., 1995b). Thus, COOH-M-OH may be the ring-opened metabolite that causes the hematotoxic effects of administered MUC, and it may play a role in causing the toxic effects of benzene exposure.

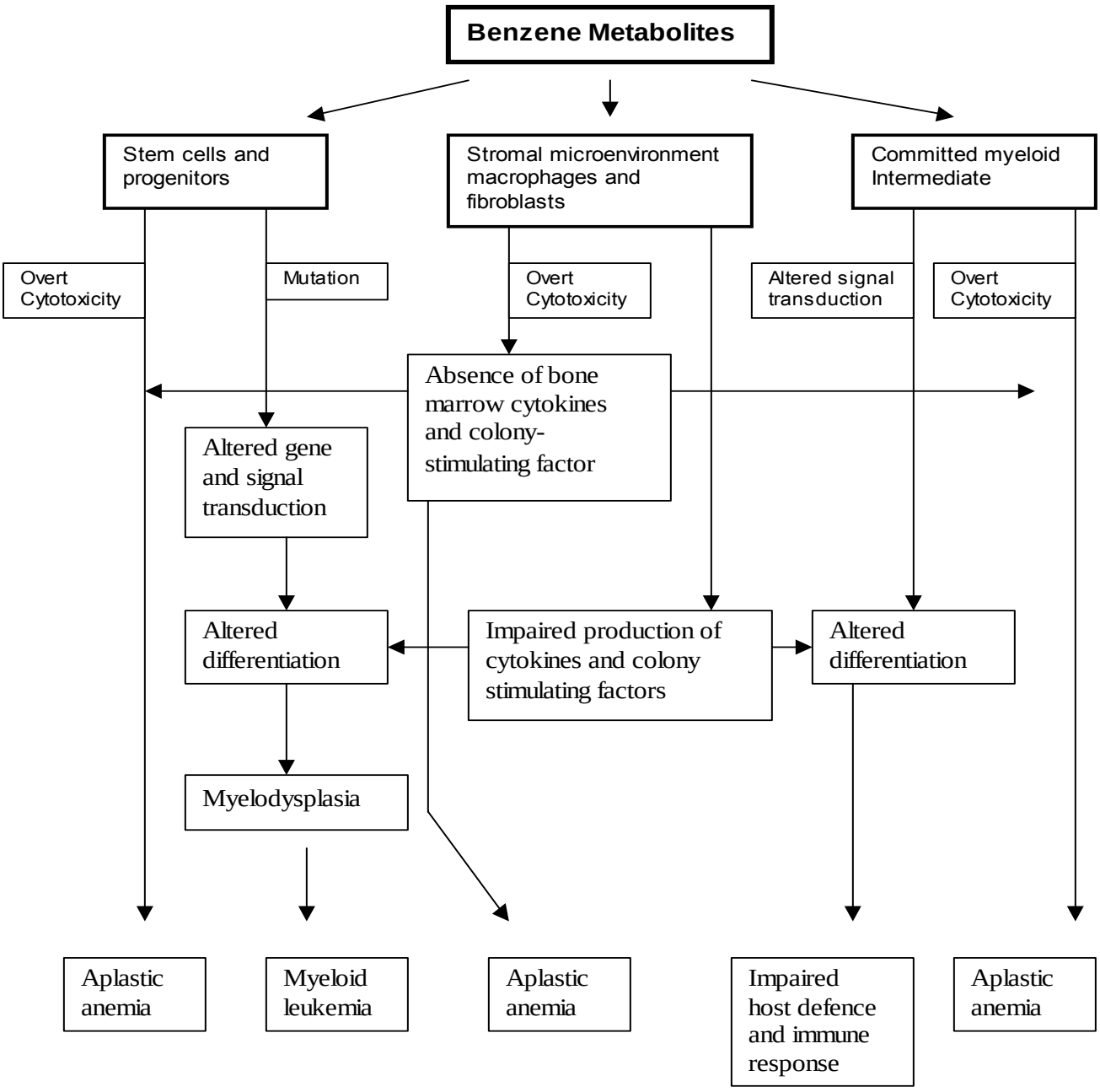
It should be noted that many of the metabolites that are thought to contribute to the toxic effects of benzene (e.g., phenol, catechol, MA) have numerous exogenous and endogenous environmental sources, and thus, may complicate any dose-response relationship calculation, especially at environmental doses (US EPA, 1998; RIVM, 2001).

Metabolism and Toxicodynamics

Overall, the altered function of different bone marrow cell populations could result in the manifestation of different types of toxicity (e.g., aplastic anemia or leukemia), possibly representing a continuum of effects, from minor to severe. It is already recognized that persistent cytopenias and other blood dyscrasias, including dyserythropoiesis, dysgranulopoiesis and dysmegakaryopoiesis, frequently precede the onset of leukemia in patients developing acute myeloid leukemia (AML) following exposure to benzene or

alkylating agents (Irons and Stillman, 1996). The overall toxicodynamic effects that are possible due to the identified pathways of benzene metabolism have been schematically represented in Figure 3.3. Briefly, the evidence suggests that benzene metabolites interfere with (1) stem cells and progenitors; (2) stromal microenvironment macrophages and fibroblasts; and/or (3) committed myeloid intermediates. Eventually the adverse effects, such as myeloid leukemia, impaired host defence and immune responses, and aplastic anemia, are possible. While concurrent events have been identified in various toxicodynamic investigations, the overall contribution of each metabolite to the observed toxicity pattern, along with dominant pathways at environmental exposure concentrations, remains not well understood.

Figure 3.3: Schematic illustration indicating the various paths by which benzene metabolites may affect bone marrow cell populations, which may result in the induction of aplastic anemia, leukemia and immunotoxicity. Sources: US EPA 1998; Trush *et al.*, 1996.



3.1.4 Elimination

The metabolic fate of inhaled benzene in humans was addressed by demonstrating that at least a percentage of the absorbed benzene may be excreted in the urine as sulfate- or glucuronide-conjugated phenolic or MA compounds (Nomiyama and Nomiyama, 1974). At the exposed benzene concentrations of 166-198 mg/m^3, respiratory uptake was considered to be approximately 47%, and respiratory excretion for the 4-hour exposure period was approximately 17%. These values were in reasonably good agreement to those obtained in an earlier study by Srbova and colleagues (Srbova *et al*., 1950) who observed a respiratory excretion of retained benzene between a 16.4-to-41.6% range, across a 7-hour exposure period.

In general, insufficient data exist to unequivocally assign one elimination route or another as being of primary importance when human beings are exposed to benzene via inhalation. For example, Sherwood (1988) employed a single human subject who was alternately exposed to either 20 mg/m^3 benzene for 8 hours, or 316 mg/m^3 for 1 hour, to monitor the kinetics of benzene elimination. Here, the authors were able to demonstrate that a greater proportion of the total dose was excreted in urine rather than via expiration. These results also showed that urinary excretion of phenol-conjugate was biphasic, with an initial rapid excretion phase followed by a slower excretion phase. The importance of urinary excretion was also emphasized by the work of Inoue and colleagues (Inoue *et al*., 1986), which showed a good correlation between urinary phenol levels and benzene exposure across a relatively wide exposure concentration range of 3.2-640 mg/m^3.

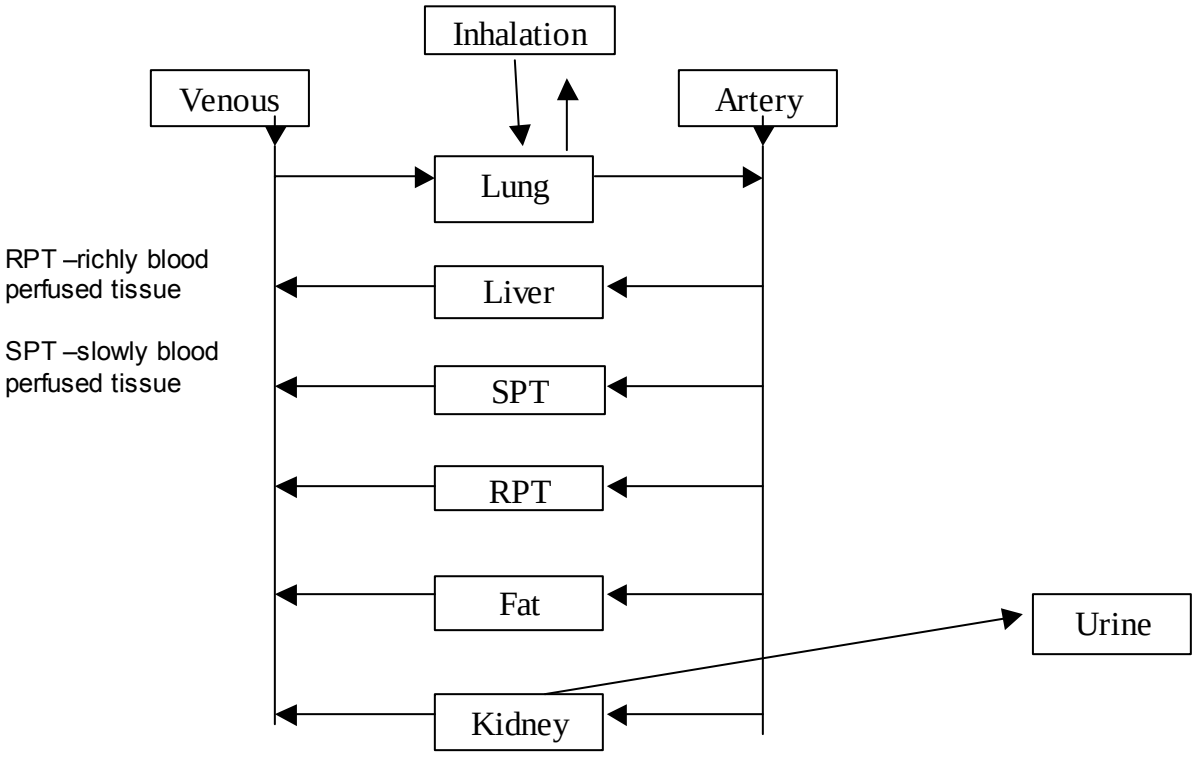
Occupational exposure and cross-sectional studies have also pointed to the appearance of benzene metabolites in urine as a consequence of exposure to such agents as side stream tobacco smoke (Bartczak *et al*., 1994) and gasoline vapours (Lagorio *et al*., 1994), or when the urine content of smokers versus non-smokers was compared (Kok and Ong, 1994; Melikian *et al*., 2002). Popp *et al*. (1994) detected muconic acid and S-phenyl-N-acetyl cysteine levels in the urine of car mechanics at levels that correlated with levels of the compound in the bloodstream and the breathing zone. These observations support the earlier suggestion by Ghittori *et al*. (1993) that benzene and its metabolites in the urine may be an important biomarker of occupational exposure to the compound.

An accumulation of experimental data in laboratory animals has shown an essentially similar pattern of benzene elimination and excretion as in human beings. In broad terms, this is characterized by the release of unchanged compound in the breath and the appearance of metabolites in the urine. These studies are reviewed by ATSDR, (2007) in detail.

3.1.5 PBTK Models

Despite decades of research on the toxicokinetics of benzene, gaps in understanding remain, especially in determining how the described findings are relevant to environmental exposures. As a result, physiologically based toxicokinetic (PBTK) models can be used to address some of the uncertainty of extrapolating from animal experimental data to a hypothetical near environmental human exposure dose. A schematic of PBTK model can be seen in Figure 3-4, where a 4-compartment model has been schematically described. A detailed description of the PBTK model for benzene is presented in Appendix A.

Figure 3.4: Schematic flow diagram of a PBTK model. A detail discussion of PBTK models for benzene appear in Appendix A

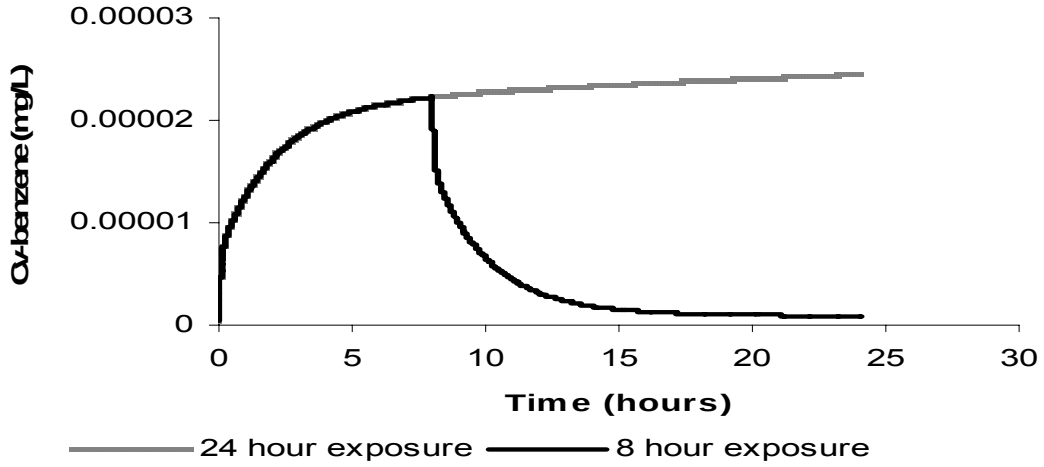


Potential advantages of the PBTK approach reside in its ability to quantitatively address interspecies differences, and to take into account the non-linearity of biological processes when extrapolating outside the range of available experimental data, which may be a significant issue, considering the metabolism of benzene. However, some limitations of the PBTK approach include the accuracy and/or validity of the estimates that constitute the inputs to the model, and in the oversimplification that is inherent to the model itself, in terms of a small number of physiologically-relevant and defined sub-

compartments. Suffice to say, a number of PBTK models have been developed and have attempted to coherently fit the available data, which, in turn, has helped to refine the specific experimental approaches performed in order to fill the gaps in the understanding of the mechanism of benzene toxicity. However, although current PBTK models may provide insights about putative toxic metabolites and potential biochemical mechanisms, the current published models are insufficiently refined to allow the prediction of human metabolism accurately. This is further complicated due to the advent of the complex low dose benzene metabolism events, explained in more detail in Section 3.8 and throughout Section 5. A survey of the published PBTK models for benzene, and a MOE PBTK model reconstruction in an attempt to take such low dose complications into account, is presented in depth in Appendix A. As an example of the usefulness of PBTK models, discussions of: how such models aid in explaining the likelihood of chemical interaction affecting metabolism at environmental exposure concentrations; the understanding of steady-state kinetics and benzene clearance from human circulatory system; and, how much models and the mixture data aid in explaining the likelihood of chemical interaction affecting metabolism at environmental exposure concentrations, are presented below.

The results of the benzene PBTK model simulation is shown in Figure 3.5. Deterministic human benzene PBTK model has been re-constructed (Haddad *et al*., 1996; algorithms found in Appendix A) and used for simulation of benzene exposure at an environmental exposure concentration. This model was used to calculate the internal dosimetry of benzene in the absence of any chemical interactions that may occur in the environment. A steady inhalation input concentration of 4.5 µg/m^3 was maintained for 24 hours of simulated exposure (Figure 3.5). Venous blood benzene concentration near steady state was achieved in about 8 hours of exposure. However, there is residual increase in venous blood benzene concentration until full duration of benzene exposure.

Figure 3.5: Time profile of internal dosimetry of benzene at the mixed venous level (Cv – mg/L) during a 24 hour and a 8 hour benzene exposure simulation at concentration of 4.5 µg/m^3.



Benzene concentration rapidly reaches an apparent steady state at about 5 to 6 hours. After the exposure is terminated at 8 hours, most of the benzene is cleared from the venous circulation within 5 to 6 hours (Figure 3.5). This shorter-term exposure is typical for 8 hour industrial shift, whereas for the general environmental condition, continuous exposures are more likely to occur. Peak benzene metabolite concentrations are achieved within the first hour of exposure and there is a sharp decrease in the metabolite concentration after the exposure is discontinued (Turteltaub and Mani, 2003). Thus in an occupational environment, which only requires or allows 8 hours of exposure, the metabolites will tend to be depleted. Conversely, in a situation of more general environmental-relevant exposure with continuous exposure throughout the year, the plasma metabolite concentration should accordingly be continuously expected to be near steady state. The presence of metabolites is linked to carcinogenesis due to benzene exposure. Hence, at environmental exposure doses, the risk of continuous peaking of metabolite concentration is high. This suggests a possibility of an underestimation of risk which would happen when occupational data are used to extrapolate the risk for the general population.

The co-exposure to many chemicals in the environment may lead to toxicokinetic interactions, thus resulting in an altered dose-response, and hence, the apparent toxicity (Mumtaz *et al*., 1993; Mumtaz *et al*.,1994; Yang, 1994; Haddad *et al*., 2001; Jang *et al*., 2001; Dennison *et al*., 2003; Dennison *et al*., 2004; Mumtaz *et al*., 2004; Wharfe *et al*., 2004; Dennison *et al*., 2005a; Dennison *et al*., 2005b; Yang *et al*., 2005). Toxicokinetic interactions of chemical mixtures certainly influence the toxic effects of any single chemical if the interactions are at all synergistic, antagonistic or competitive (Yin *et al*., 1987a; De Rosa *et al*., 2004; McCarty and Borgert, 2006). These interactions will depend on the chemical mixture constitution, chemical quantity (Feron *et al*., 1998; Feron and Groten, 2000; Pohl *et al*., 2003; Dennison *et al*., 2004), and will thus influence the dose-response slope (Altenburger *et al*., 2003; Gennings *et al*., 2005; Rider and LeBlanc, 2005). Metabolic interactions always take place above an interaction threshold. Interaction threshold is defined as the exposure level only above which an interaction phenomenon would occur (Dennison *et al*., 2004). The inhibitory interaction threshold can be measured by the inhibitory constant (Ki) and is a determinant in calculating the internal dosimetry of a chemical due to mixture interaction. Benzene interaction with toluene, ethyl benzene, xylene, and dichloromethane (i.e., common petrochemical products often present as significant co-exposures with benzene), have been shown to be CYP2E1 competing VOCs, and the kinetic constants have been established (Haddad *et al*., 2001) for an appropriate toxicokinetic analysis of benzene.

As some benzene metabolites are considered to cause carcinogenesis in human, the internal dosimetry of the metabolites becomes important. The influence of chemical interaction in terms of the amount of benzene metabolized has been shown in Table 3.1 along with the validated inhibitory kinetics constants. This table is adapted from Haddad *et al*. (2001). The change in calculated cancer risk, due to a decrease in amount of benzene metabolized because of metabolic competition (Table 3.1), highlights the limitation of using the higher dose ranges of the epidemiological studies

as direct representatives of benzene metabolism at lower, environmental exposure doses.

Table 3-1: Effect of toxicokinetic interactions on the cancer risk level associated with benzene (B) present in mixtures along with dichloromethane (D); toluene (T); ethylbenzene (E) and m-xylene. (These metabolic data and following risk calculations are based on PBTK Modeling of benzene in humans carried out by Haddad et al., 2001)

Exposure Concentration (ppm)					Amount benzene metabolized (mg)		Change in cancer risk*
D Ki = 0.08	B	T Ki = 0.22	E Ki = 0.63	X Ki = 0.23	mixture	B single	B (due to mixture)
50	0.5	50	100	100	1.19	3.14	0.38
25	0.5	25	50	50	1.93	3.14	0.61
16	0.5	16	33	33	2.39	3.14	0.76
12.5	0.5	12.5	25	25	2.61	3.14	0.83
10	0.5	5	40	20	2.57	3.14	0.82
10	0.5	10	10	10	2.93	3.14	0.93

Ki – enzyme inhibition constant (chemical mixture effect)

***- Ratio between amount benzene metabolised** $\left(\frac{mixture}{benzene(alone)}\right)$

Briefly, the toxicokinetic interferences from toluene, xylene, ethyl benzene and dichloromethane have been tabulated in Table 3-1. The table accounts for the amount of benzene metabolized when exposed either as a single chemical or as mixture with varying mixture concentration. The ratio between the amounts of benzene metabolized with and without the chemical mixture during 24 hour exposure, shows that there is a significant amount of reduction in the amount of benzene metabolized, hence there should be a reduction in the toxic metabolized formed in petrochemical industries where chemical mixture are often encountered. The consequences, and complications, of such interactions are elaborated further in Section 5 – that is, its applicability at industrially-relevant doses.

3.2 Acute Exposure

Acute exposure of benzene due to inhalation or oral exposure produces CNS effects in both humans and animals, which clear rapidly upon exposure cessation (Carpenter *et al*., 1944; Cornish and Ryan, 1965; Tauber, 1970; Thienes and Haley, 1972; Midzenski *et al*., 1992; ATSDR, 2007). Inhalation of 800 to 1600 mg/m^3 produces vertigo,

drowsiness, headache, and nausea (Duarte-Davidson *et al*., 2001), whereas, higher concentrations (4800 mg/m^3) lead to euphoria followed by giddiness, headache, nausea, staggered gait and, with continued exposure, unconsciousness (Sandmeyer, 1981). Short-term exposures up to 9600 mg/m^3 can be tolerated for 0.5-to-1 hours. However, exposure to massive concentrations of 64,000 mg/m^3 or higher can be fatal within 5-to-10 minutes (Duarte-Davidson *et al*., 2001).

While systemic toxicity of benzene mainly targets the nervous and the hematopoietic systems, ventricular fibrillation has been proposed as the cause of death in some human poisoning reports (Winek and Collom 1971; Avis and Hutton 1993).

3.3 Subchronic and Chronic Exposure

The majority of the longer-term exposure studies have focussed on the carcinogenic potential of benzene, with the development of leukemia being the toxicological endpoint of concern. However, a few subchronic and chronic benzene exposure studies which examine toxic effects other than carcinogenesis have been identified, and their conclusions are briefly highlighted below.

Intermediate-duration inhalation and oral exposure to benzene induce neurological effects in animals including reduced limb grip strength, behavioural disturbances, and changes in brain levels of monoamine neurotransmitters and acetylcholinesterase (Dempster *et al*., 1984; Hsieh *et al*., 1988; Li *et al*., 1992; Frantik *et al*., 1994).

Hematotoxicity is the most noted and characteristic systemic effect resulting from subchronic and chronic benzene exposure in humans and animals. All of the major types of blood cells are susceptible. A common clinical finding is cytopenia, which is a decrease in various cellular elements manifested as anemia, leucopenia, pancytopenia or thrombocytopenia in humans (Fauci *et al*., 1998). Prolonged exposure to benzene can cause severe damage to the bone marrow involving cellular aplastic anaemia, which is characterized by reduction of all cellular elements in the peripheral blood and in bone marrow. Benzene-induced aplastic anemia can progress to AML (Doskin, 1971; Aksoy *et al*., 1974; Aksoy 1980; Rozen *et al*., 1984). Adverse hematological effects begin to appear in animals at benzene concentrations of 32-324 mg/m^3 and above. Immunological changes in humans and animals due to benzene exposure appear to be largely related to decrease in circulating leukocytes and the ability of lymphoid tissue to produce the mature lymphocytes necessary to form antibodies (ATSDR, 2007).

Pancytopenia and clastogenic aplastic anemias are common non-cancer hematological disorders due to benzene exposure (Bruckner and Warren, 2001). Patients with hypocellular myelodysplastic syndrome also have pancytopenia and hypocellular bone marrow. Clinically, blood smears however may show the presence of immature granulocytes or nucleated red cells. The few myeloid elements in the marrow have dysplastic changes, and the marrow karyotype may show a clonal abnormality. Differential diagnosis is difficult when the dysplastic changes are subtle. Hypocellular acute leukemia can be misdiagnosed as aplastic anemia (Castro-Malaspina and O'Reilly, 2001).

Musculoskeletal effects were observed in workers from a steel plant in Sao Paulo, Brazil, who presented cases with neutropenia due to benzene exposure. Sixty percent of the workers had non-specific clinical complaints, such as myalgia (Ruiz *et al*., 1994). Aksoy *et al*. (1972) reported enlarged livers in workers chronically exposed to benzene at air concentration ranging from 486-2100 mg/m^3.

Oral data in subchronic and chronic-duration animal studies show that loss of blood elements occurs following exposure to benzene in drinking water or by gavage at doses as low as 8-25 mg/kg/day (ATSDR, 2007).

Dermal effects due to benzene exposure are limited to skin irritation, possibly leading to second degree burns and hemorrhagic respiratory tissue (Midzenski *et al*., 1992; Avis & Hutton, 1993).

In conclusion, it should be stated that as a number of the acute, sub-chronic and chronic non-cancer toxic endpoints involve hematological issues, the Ministry cannot dismiss the possibility that such adverse effects may represent early precursor events in leukemia pathology.

3.4 Developmental and Reproductive Toxicity

Overall, very few studies were identified involving reproductive or developmental effects from exposures to benzene. However, lately there has been a growing appreciation of the developmental and reproductive effects of chronic benzene exposure. While two generational studies could not be identified, the evidence of toxic effects on developmental and the reproductive system is highlighted below.

Adequate reproductive and developmental quantitative toxicological data on benzene are essentially limited to results of inhalation studies in animals. There is suggestive evidence of benzene-induced testicular effects (e.g., atrophy/degeneration, decrease in spermatozoa, increase in abnormal forms), particularly in mice following intermediate duration exposure to 970 mg/m^3 (Wolf *et al*., 1956; Ward *et al*., 1985). Results of developmental toxicity studies indicate that inhalation exposure to high levels of benzene is fetotoxic and maternally toxic in several species, as shown by decreased fetal weight and/or minor skeletal variants. Fetotoxic effects in rodents occurred at benzene levels ≥ 150 mg/m^3 (Green *et al*., 1978; Murray *et al*., 1979; Tatrai *et al*., 1980a; Tatrai *et al*., 1980b; Kuna and Kapp, 1981; Coate *et al*., 1984; Ungvary and Tatrai, 1985), although there was evidence of transient hematopoietic anomalies in fetuses and offspring of mice exposed to 15-60 mg/m^3 benzene (Keller and Snyder 1986; Keller and Snyder, 1988). There was an increase in the maternal liver weight of pregnant rats exposed to benzene at a concentration of 450 mg/m^3 (Tatrai *et al*, 1980a; Tatrai *et al*, 1980b). Kidney effects are limited to individual cases, and data are very scarce (ATSDR, 2007).

Gynecological disturbances in women workers exposed to benzene have been recorded. In two separate studies involving petrochemical exposure to female workers, menstrual cycle disturbances have been demonstrated (Michon, 1965; Mukhametova and Vozovaya, 1972). While in the Michon (1965) study benzene exposures were estimated to be less than 800 µg/m^3, it is noted that in both these studies the benzene

exposure calculations were poor. Stucker *et al*. (1994) examined the reproductive competence of male workers in organic chemical factories, where measure for such competency is the incidence of spontaneous abortion in their counterparts. Two benzene exposure groups (< 16 mg/m^3 and ≥ 16 mg/m^3) were identified. In both groups, there was no increase in abortive response during pregnancies when compared to the control populations. Overall, the available data regarding benzene exposure on developmental effects remain inconclusive (ATSDR, 2007).

Evidence of age-related differences in susceptibility to benzene has not been identified (ATSDR, 2007). *In utero* exposure studies to benzene in animals show no difference in haematological changes than that in adult animals (Keller and Snyder 1986; Keller and Snyder, 1988; Corti and Snyder 1996). Occupational exposure of parents may play a role in childhood leukemia (Shaw *et al*., 1984; Shu *et al*., 1988; Buckley *et al*., 1989; McKinney *et al*., 1991). However, none of these studies are directly related to AML, nor indicate whether children are at greater risk than adults. A recent study examined whether census tracts with the highest estimated levels of benzene and 1,3-butadiene have higher incidence rates of childhood lymphohematopoietic cancer, in and around Houston, Texas (Whitworth et al., 2008). The authors detected a statistically significant trend of increasing incidence rates with increasing estimated levels of benzene for all leukemias combined ($p = 0.03$) and a borderline significant trend for AML ($p = 0.06$). Similarly, significantly trends of increased rates of all leukemia with 1,3-butadiene were also detected ($p = 0.01$). However, due to the high correlation between estimated ambient air levels of benzene and 1,3-butadiene, the authors were unable to confirm a clear association between the benzene exposure and occurrence of AML. Overall, there was no information available that directly relates risk of childhood AML to benzene exposure (ATSDR 2007).

Exposure of fathers to benzene and other solvents before conception are post-natally associated with increased risk of childhood leukemia (McKinney *et al*., 1991; Buckley *et al*., 1989; Lowengart *et al*., 1987). However, none of these studies have quantitative estimates of benzene, or mechanistic explanations for such effects.

Interestingly, CYP2E1 was not detected in the human fetus and the concentration of this enzyme increases only postnatally (Vieira *et al*., 1996; McCarver *et al*., 2003). Due to reduced metabolic capacity, fetus and neonates may be at reduced risk of benzene leukomogenesis (ATSDR, 2007). Regardless, there are limited studies regarding the tissue response (toxicodynamic) of benzene exposure during childhood.

3.5 Genotoxicity

The pathways of benzene metabolism include formation of covalent adducts, genotoxicity, oxidative stress and inhibition of cytokine formation. Benzene metabolites form covalent adducts with both cell proteins and DNA. Genotoxic damage, initiated by benzene metabolite-mediated DNA adducts formation, is thought to be an essential aspect of benzene-induced carcinogenesis. Indeed, it is will be further discussed in Section 5, the Ministry cannot dismiss such damage as being preclinical biomarkers of AML.

Gene mutation assays in bacteria or *in vitro* mammalian cell systems exposed to benzene and its metabolites revealed inconclusive results (Kaden *et al*., 1979; Seixas *et al*., 1982; Ashby *et al*., 1985; Oberly *et al*., 1984, Glatt *et al*., 1989; Oberly *et al*., 1990). The Ames test with *Salmonella typhimurium* – a reverse mutation test – has resulted negative for both benzene and its metabolites (De Flora *et al*., 1984). However, reverse mutation test for histidine (Glatt *et al*., 1989) and azaquanine (Kaden *et al*., 1979; Seixas *et al*., 1982) with *Salmonella typhimurium* has resulted positive for benzene metabolites. Additionally, Ward *et al*. (1992) reported dose-related increases in mutations at the *hprt* locus in lymphocytes of CD-1 mice exposed to benzene (40, 100, and 1,000 ppb) by inhalation for 6 weeks (22 h/day, 7 days/week). There is strong evidence to support the mutagenic potential of benzene based on a number of different *in vivo* assays, as well as the results of a number of animal and human studies (see Section 3.7 for Dean, 1978; Dean, 1985; Nilsson *et al*., 1996; Stronati *et al*., 2004; Sul *et al*., 2005; and other publications discussed below). The literature on the genotoxic effects of benzene is extensive, with more than 220 publications of original data (ATSDR, 2007).

The metabolites of benzene have been shown to covalently bind to DNA, where a number of short-term in vitro assays has been conducted examining their mutagenic potentials. These assays have examined a number of different endpoints, such as bacterial DNA repair, bacteriophage induction, sister chromatid exchange, chromosomal aberrations and point mutations. While there is paucity in confirmed point mutations in human *in vivo* tests, the results of some of the key genotoxic studies are highlighted below. Furthermore, the reader is also referred to ATSDR (2007) for a detailed analysis of benzene genotoxicity.

Benzene is considered a clastogen, causing chromosomal aberrations *in vitro* and *in vivo* (Greiner *et al*., 2000; Galm *et al*., 2006; Bollati *et al*., 2007). Cytogenetic effects observed from *in vivo* animal studies include chromosome and chromatid aberrations, sister chromatid exchanges, micronuclei, DNA cross linking, DNA adduct formation and alteration in DNA repair (Anderson and Richardson, 1981; Siou *et al*., 1981; Toft *et al*., 1982; Au *et al*., 1991; Erexson *et al*., 1986; Fujie *et al*., 1992; Ward *et al*., 1992; Kolachana *et al*., 1993; Au *et al*., 2002). For example, a study by Liu and colleagues (Liu *et al*., 1996) found that both medium (40-200 mg/m^3) and high (>200 mg/m^3) concentrations of benzene resulted in significantly increased concentrations of the oxidative DNA adduct, 8-hydroxydeoxyguanosine, in workers at a shoe factory. Additional studies report similar molecular events taking place at much lower exposure concentrations, both in animal and human studies. For example, Hedli and colleagues (Hedli *et al.,* 1996) investigated DNA adduct formation from the benzene metabolites hydroquinone and 1,2,4-benzenetriol, at concentrations in the order of picomoles. In combination with investigations of the effect of these two metabolites on cell differentiation in a hematopoietic system (isolated mice liver), the authors described the formation of DNA adducts in human promyelocytic leukemia cells with hydroquinone, but not with 1,2,4-benzenetriol. Both metabolites, however, inhibited retinoic acid induced maturation of human promyelocytic leukemia cells to granulocytes. Thus, DNA adduct formation may be an important effect of some of the benzene metabolites.

The association between benzene exposure and the appearance of structural and numerical chromosomal aberrations in human lymphocytes further supports benzene as a human clastogen. Several lines of evidence also indicate that benzene is genotoxic in humans under occupational exposure conditions (Ding *et al*., 1983; Sasiadek *et al*., 1989; Yardley-Jones *et al*., 1991; Major *et al*., 1992; Eastmond, 1993; Tompa *et al*., 1994). However, these studies lack good exposure monitoring data, involved multiple chemical exposures, and were often poorly designed, with inappropriate control groups (ATSDR, 2007). Benzene may also produce oxidative stress in the target tissues. The benzene metabolite p-benzoquinone is highly reactive and can deplete cellular levels of GSH (Brunmark and Cadenas, 1988). Benzene metabolites can also be involved in redox cycling, resulting in the production of reactive oxygen species that can also react with macromolecular components (Rao and Snyder, 1995).

Other major metabolites of benzene, muconic acid and hydroquinone, form both DNA and protein adducts. These bioactive forms of benzene are distributed in organs such as liver, bone marrow, spleen, and kidney (Creek *et al*., 1997; Turteltaub and Mani, 2003). Reactive electrophiles, including benzene oxide, 1,4-benzoquinone, and 1,2-benzoquinone, are capable of reacting with blood proteins to produce adducts (Lin *et al.*, 2007). Creek *et al*., (1997) showed that DNA adduct formation via benzene metabolites following i.p. administration of ^{14}C-benzene was linear over a dose range spanning eight orders of magnitude in B6C3F1 mice. Briefly, benzene was administered to male B6C3F1 mice over a dose range of 700 pg/kg to 500 mg/kg bw. A dose-dependent linear DNA adduct formation was observed from 700 pg/kg bw until 16 mg/kg bw. For comparative purposes, a 700 pg/kg bw mouse with an assumed benzene absorption rate of 50%, a body weight of 25g, and a respiration rate of 1.5 L/hour (US EPA, 1994) would be equivalent to an environmental concentration of 0.97 µg/m^3 benzene. At doses greater than 16 mg/kg-bw, however, adduct formation was non-linear. This corresponds very closely to the metabolic saturation level of 15 mg/kg bw observed by Sabourin *et al*. (1987), and will be discussed in depth in Section 5. Liver DNA adduct levels peaked at 0.5 hours, and the bone marrow DNA adduct level peaked between 12 and 24 hours. Overall, these data indicate that adduct formation is linear in the range of benzene concentrations which may be environmentally relevant. It is noted that while DNA adduct formation does not prove the initiation of carcinogenesis – a *toxicodynamic* event – it still is a manifestation of the *toxicokinetic linearity* of metabolite formation.

Indeed, while the mechanism by which benzene exerts its carcinogenic effects remains not fully understood, it is considered that mutations induced by benzene-DNA adducts may play a role. Another example of the genotoxic effect of the benzene metabolite para-benzoquinone was observed following reaction *in vitro* with DNA, where it formed four major adducts, two of which are 2'-deoxyguanosine 3'-monophosphates (dGp). As discussed above, reaction of the benzene metabolite hydroquinone and DNA results in adduct formation, which resembles dGp. Interestingly, it has been hypothesized that it is this dGp adduct formation that may play an important role in the mutagenicity of benzene (Gaskell *et al*., 2004; Gaskell *et al*., 2005a; Gaskell *et al*., 2005b). In one study, *in vitro* mutagenic assays were performed using the benzene metabolites hydroquinone and p-benzenoquinone (Gaskell *et al*., 2004). This experiment was

conducted using the supF forward mutation assay. The metabolites treated plasmid (pSP189) containing the supF gene was replicated in human Ad293 cells before being screened in indicator bacteria. p-Benzoquinone treatment at concentrations of 5, 10 and 20 mM showed increased mutation frequencies and increased incidence of adduct formed (Figure 3-6). Taken together, these experiments may represent a possible link between adduct formation and mutagenecity, thus aiding in the understanding of the role of metabolites in benzene toxicity.

Nakayama *et al*. (2000) conducted an *in vitro* assessment of the mutations induced by p-benzoquinone in human and mouse cells. The authors found that p-benzoquinone induces mutation in humans and mouse cells at similar frequencies, but with different types of mutagenesis. Specifically, the observed proportion of tandem base pair mutations was significantly lower in human cells than in mouse cells. However, the G: C → C: G transversion proportion was significantly higher in human cells than in mouse cells. These findings reveal that *p*-benzoquinone-induced DNA damage in human and in mouse cells may be processed differently.

Similarly, Turteltaub and Mani (2003), hypothesize that the differences in the capacity of various species of mice and rats to metabolize benzene affect macromolecular adduct formation, and the amount of macromolecular damage is related to benzene's ability to cause cancer and other blood disorders.

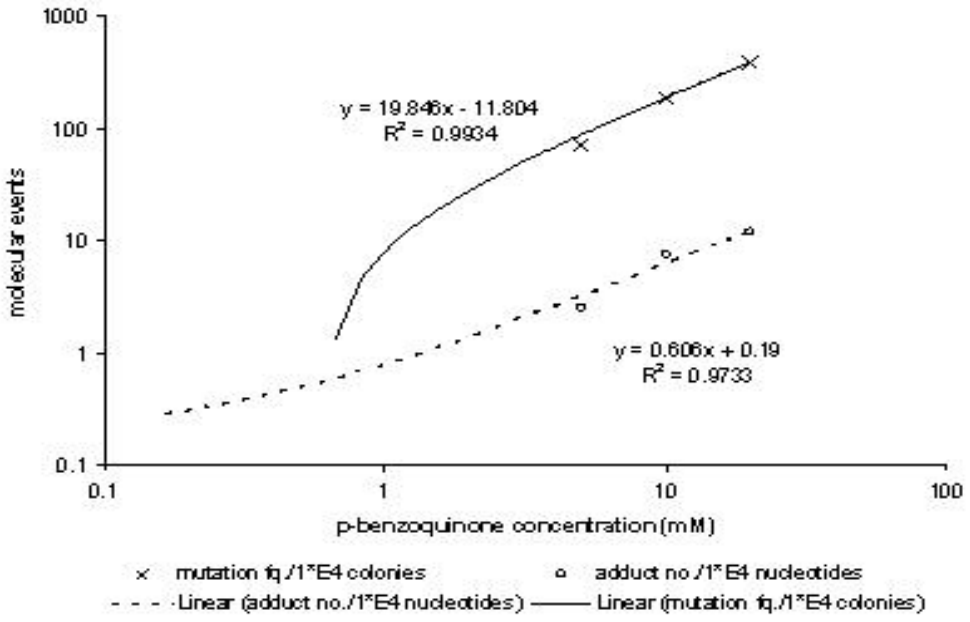


Figure 3.6: Adduct numbers and mutation frequencies observed due to varied concentrations of p-benzoquinone exposure in supF mutation assay. Source: Gaskell et al., 2004.

In several studies, increased levels of chromosomal aberrations in peripheral blood lymphocytes were correlated with a heightened risk of cancer, especially hematological malignancies (Holeckova *et al*., 2004). Zhang et al., (2002) reviewed the pattern of the chromosomal aberrations in patients with AML without a history of benzene exposure and compared to chromosomal aberrations in benzene exposed individuals. Zhang *et al* (2002) specifically, in their review of chromosomal aberrations detected in humans exposed to benzene was taken from various studies over the previous 40 years, the concentration ranges used were from 0.02 ppm (64 µg/m^3) to 500 ppm (1600 mg/m^3). The duration of exposure varied from 0.1 year to more than 40 years and the number of subject tested ranged from 100 to 200. Based on this review Zhang *et al*. (2002) suggest that chromosomal aberrations may be a predictor of future leukemic risk. Holeckova *et al*., 2004 further concluded that the frequencies of chromosomal aberrations as well as micronuclei can be used as biomarkers of effects. It seems almost certain that chromosome specific aneuploidy and translocation play key roles in the development and progression of leukemia, as well as many other cancers. Therefore, chromosome specific aneuploidy with higher sensitivity to benzene exposure would be a useful biomarker for leukemia risk (effect) of benzene (Holeckova *et al*., 2004).

Formation of benzene metabolites and induction of DNA-repair capacity was assessed as a biomarker of preclinical effect (Chanvaivit *et al*., 2007). Here, the mean individual benzene exposure of laboratory workers 78 µg/m^3 (24.4 ± 5.82 ppb; n=31) and that of gasoline service attendants 360 µg/m^3 (112.4 ± 13.92 ppb; n = 31) were significantly higher than in controls 4.45 µg/m^3 (1.39 ± 0.17 ppb; n = 34). Characterizations of dose-response were carried out (Figure 3.7) and the parameters include urinary tt-muconic acid, blood benzene, incidence of dicentrics, and frequency of deletion at the blood DNA level.

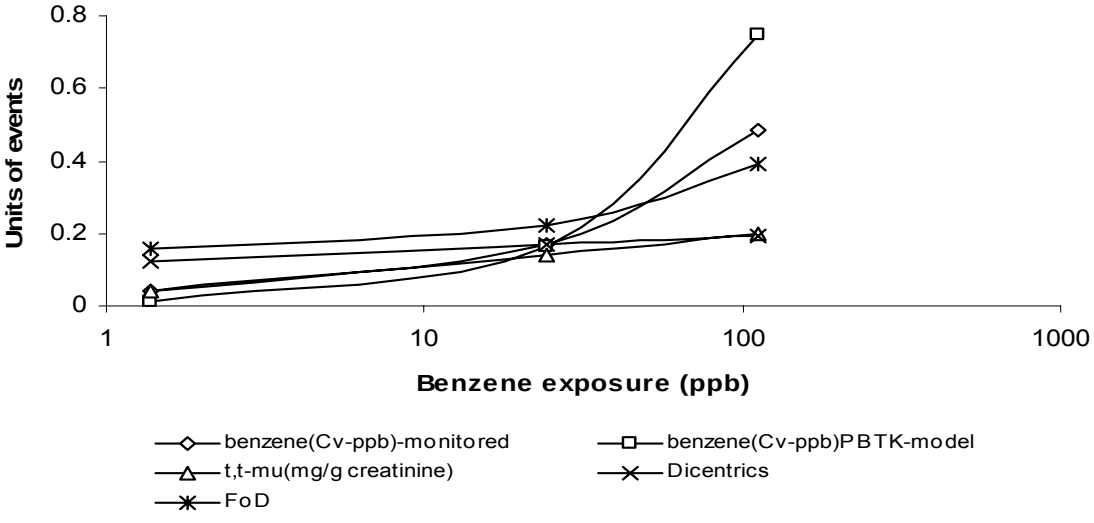


Figure 3.7: Human benzene biochemical parameter and chromosomal event (Source: Chanvaivit et al., 2007)

Blood benzene and the metabolites, tt-muconic acid concentrations were higher in tests in a dose dependent way than in controls. Urinary metabolite concentration was proportional to the amount of metabolite formed in the body. Hence this is an indication of a dose-response of metabolite formation at a dose range of 4.45 to 360 µg/m^3. Metabolite (tt-muconic acid) formation shows a linear trend within the range and could not support any threshold.

As a biomarker of preclinical effect, DNA-repair capacity was assessed by use of the cytogenetic challenge assay (Chanvaivit *et al*., 2007), where chromosomal aberrations in the peripheral lymphocytes were assessed after challenging blood cultures with gamma radiation. A significantly lower DNA-repair capacity, determined as dicentrics in laboratory workers (0.17 / metaphase cell) and in gasoline service attendants (0.19 / metaphase cell) compared with controls (0.12 / metaphase cell, $p<0.001$), was observed. The frequency of genetic deletions in laboratory workers (0.22 / metaphase cell) and gasoline service attendants (0.39 / metaphase cell) were significantly higher than in control workers (0.16 / metaphase cell $p<0.01$ and $p<0.001$ respectively). A dose-response based increase in radiation-induced dicentrics and deletions is possibly due to a lower DNA-repair capacity in benzene exposed workers. The authors suggested that lower DNA-repair mechanisms at low doses are early biomarkers of effect, and also suggest differential genetic susceptibility amongst the human population. The authors conclude that even at relatively low levels of benzene exposure, workers may have increased risk for genotoxicity that is due in particular to a decrease in DNA-repair capacity.

However, the role of DNA adducts and benzene metabolite-generated mutations in the development of cancer following benzene exposure remains not fully understood. For example, it has been noted that the carcinogenic effect observed at occupational levels of benzene cannot be explained by just genotoxic mechanisms – that is, blood and bone marrow toxicity may be involved or even may be conditional (reviewed in RIVM, 2001). Specifically, the fact that in experimental animals carcinogenic inducing exposures clearly exceeded those exposures reported to be hematotoxic inducing, and the fact that benzene does not appear to interact with DNA directly and thus has limited potential to directly induce gene mutations (reviewed in RIVM, 2001), demonstrates that a mechanistic understanding linking the toxicokinetic and toxicodynamic effects of benzene has not been fully developed. However, recent findings – discussed in the following sections – have begun to shed light on such links.

Experiments in rodents have provided consistent evidence from a number of studies that benzene exposure causes increased frequency of micronucleated cells (ATSDR, 2007). Micronuclei also are seen in human cells exposed *in vitro* to various metabolites and combinations of metabolites (which exhibits synergistic effects in certain combination) (Zhang *et al*., 1993; Eastmond, 1993; Yager *et al*., 1990; Hogstedt *et al*., 1991; Robertson *et al*., 1991; Marrazzini *et al*., 1994). The evidence that human exposure to benzene produces the types of chromosomal rearrangements associated with AML and MDS, such as interstitial deletions, inversions, or translocations, continues to accumulate (ATSDR, 2007). Earlier studies of patients with benzene-induced hematopoietic disorders demonstrated increased chromosome aberrations in lymphocytes and bone marrow cells (Dean, 1985). The rearrangements observed

included stable and unstable aberrations (Forni, 1971; Van den Berghe *et al*., 1979; Sarto *et al*., 1984; Aksoy, 1989; Sasiadek, 1992; Forni, 1994).

Additional epigenetic events, which are contributing steps in the benzene metabolite-induced cellular damage include numerical changes in the C-group chromosomes 6-12 and X, which have been detected in the blood and bone marrow of patients with benzene-induced myelogenous leukemia, myelodysplasic syndrome, and pancytopenia (Vigliani and Forni, 1996). Zhang *et al*. (1996) showed that the induction of aneuploidy of chromosome 9 as measured by FISH in interphase lymphocytes from benzene-exposed workers is significantly elevated, but only at high levels of exposure (>31 ppm in air). The human evidence for aneuploidy induction is also supported by *in vitro* experiments. Hydroquinone and 1,2,4-benzenetriol induce aneuploidy of chromosomes 7 and 9 in human cells (Zhang *et al*., 1994; Eastmond *et al*., 1994). Eastmond and co-workers also have reported that micronuclei containing centromeres are formed in bone marrow and spleen cells following oral benzene exposure in mice (Chen *et al*., 1994; Chen and Eastmond, 1995). Centromeres containing micronuclei are thought to be formed when a whole chromosome is lost during mitosis. Thus, considerable evidence supports the assertion that exposure to benzene produces aneuploidy in a variety of systems.

Based on the above discussions in this section, it is becoming clear that not only are mutagenic events implicated in benzene metabolites-induced cellular toxicity – epigenetic events are as well. Since the complete mechanism of leukomogenesis due to benzene and its metabolite exposure is not known, all these events should be assumed to be independent in their action. The important point of this discussion is that mutagenic events might play a key role in leukomogenesis, based on the collective evidence of the available literature.

3.6 Carcinogenicity

Benzene has long been known to be a human carcinogen, with the strongest evidence linking it with lympho-hematopoietic cancers (Duarte-Davidson *et al*., 2001). Human epidemiological-based cancer studies will be discussed in Section 3.7.

Benzene is a recognized rodent carcinogen (Gaskell *et al*., 2004). Specifically, increased incidence of lymphomas, Zymbal gland carcinoma, and other neoplasms were found in rats and mice following chronic inhalation or oral exposure (Snyder *et al*., 1980; Cronkite *et al*., 1984; Snyder *et al*., 1984; Cronkite *et al*., 1985; Cronkite *et al*., 1986; Snyder *et al*., 1988; Cronkite *et al*., 1989; Farris *et al*. 1993; ATSDR, 2007).

The pathology of tumourigenesis or hematological response like leukemia is typically based on the metabolites of benzene – hence, the analysis of the mechanism becomes important. The carcinogenic initiating mechanism of benzene involves its metabolism. These molecular actions may include formation of covalent adducts, genotoxicity, oxidative stress and inhibition of cytokine formation (Andreoli *et al*., 1997).

Benzene metabolites form covalent adducts with both cell proteins and DNA; however the role of adduct formation in toxicity is unclear. Treatments that reduce benzene toxicity also reduces adduct formation. Nonetheless, the evidence suggests that the

complexity of the toxicokinetics and toxicodynamics of the metabolites of benzene leads to the genesis of multiple varieties of cancer. However, a dose-response relationship is observed only in leukemia, and as such, leukemia is considered the critical carcinogenic endpoint. Acute Myeloid Leukemia (AML) is a typical leukemic subtype that is highly suggested as the critical endpoint due to benzene exposure and toxicity in human populations. However, other types such as Chronic Myeloid Leukemia (CML), Acute Lymphocytic Leukemia (ALL), Chronic Lymphocytic Leukemia (CLL) have also been observed in populations exposed to benzene (Wong and Raabe, 1995; Savitz and Andrew, 1997). Additionally, an increased incidence of malignant lymphomas and a variety of solid tumours were found in male and female mice exposed orally with high doses (0, 25, 50 and 100 mg/kg) of benzene for up to 103 weeks (Huff *et al*., 1989). In summary, based on mechanistic, animal studies, and other relevant epidemiological data, benzene is considered to be an animal and human carcinogen, but major species differences exist, including specific endpoints of carcinogenesis.

As mentioned in the previous section, the mechanism by which adducts formation results in bone marrow toxicity is not well established. However, a few experiments have suggested that binding of the benzene metabolites hydroquinone or p-benzoquinone to sulfhydryl groups at the active site of blood plasma protein could be responsible for the genotoxic effects of benzene. Animal and human *in vitro* studies have lead to the finding of the formation of both protein and DNA adducts from exposure to benzene. Macromolecular binding and tissue distribution of benzene metabolites have been extensively studied in different animal species and in humans. Dose-response analysis of macromolecular binding of benzene metabolites and the formation of adducts has been studied at different dose ranges in different animal species and in human epidemiological studies (Creek *et al*., 1997; Au *et al*., 2002; Cavalieri *et al*., 2002; Melnick, 2002; Qu *et al*., 2002; Rappaport *et al*., 2002a; Rappaport *et al*., 2002b; Albertini *et al*., 2003; Qu *et al*., 2003; Saieva *et al*., 2003; Turteltaub and Mani, 2003; Cavalieri and Rogan, 2004; Faiola *et al*., 2004; Gaskell *et al*., 2004; Whysner *et al*., 2004; Gaskell *et al*., 2005a; Rappaport *et al*., 2005; Waidyanatha and Rappaport 2005; Waidyanatha *et al*., 2005; Xie *et al*., 2005; Cavalieri and Rogan, 2006; Lin *et al*., 2006; Li and Yin, 2006; Lin *et al*., 2007; Ruchirawat *et al*., 2007). As an example, extensive chromosomal studies allow the conclusion that presence of benzene-induced chromosomal aberrations may be a biomarker of toxic events for leukemic risk (Forni 1996; Au *et al*., 2002). Specifically, according to Snyder and colleagues (Snyder *et al.,* 1993), benzene and its metabolites are highly clastogenic, producing chromosomal aberrations, sister chromatid exchanges and micronuclei. In several studies, increased levels of chromosomal aberrations in peripheral blood lymphocytes were correlated with a heightened risk of cancer, especially hematological malignancies (Holeckova *et al*., 2004). In fact, such chromosomal aberrations have been suggested to be a predictor of future leukemic risk (Zhang *et al*., 2002).

It is worth noting that non-regulatory research agencies, such as the International Agency for the Research on Cancer (IARC, 1987) and National Toxicology Program (NTP, 2005) also conclude benzene as a human carcinogen. Their conclusions are summarized below:

International Agency for the Research on Cancer (1987):

Evidence for carcinogenicity to humans is sufficient.

The IARC found that three independent cohort studies have found an increased incidence of acute non-lymphocytic leukemia in workers exposed to benzene (IARC, 1982; Decouflé *et al*., 1983). Another cohort study found an excess of AML among refinery workers (McCraw *et al*., 1985). A Chinese retrospective cohort study of 28,460 workers exposed to benzene compared to an unexposed population (28,257 workers) found the mortality rate from leukemia to be 14/100,000 person-years compared to 2/100,000 person-years, respectively (Yin *et al*., 1987).

Evidence for carcinogenicity to animals is sufficient.

Studies in rats and mice exposed by oral administration found that benzene induced neoplasms at multiple sites (Maltoni *et al*., 1982a; Maltoni *et al*., 1983; Maltoni *et al*., 1985; NTP, 1986). Mice exposed by inhalation exhibited lymphoid neoplasms (Cronkite *et al*., 1984; Cronkite *et al*., 1985). Rats exposed by inhalation primarily exhibited increased carcinomas at various sites (Maltoni *et al*., 1982b; Maltoni *et al*., 1982c; Maltoni *et al*., 1983; Snyder *et al*., 1984; Maltoni *et al*., 1985).

Benzene induced chromosomal aberrations, micronuclei and sister chromatid exchanges in bone-marrow cells of mice, chromosomal aberrations in bone-marrow cells of rats and Chinese hamsters, and sperm-head anomalies in mice treated *in vivo*, and also induced chromosomal aberrations and mutation in human cells *in vitro*. Benzene induced cell transformations, aneuploidy, and chromosomal aberrations in cultured cells. Benzene also induced mutation and DNA damage in some studies in rodent cells *in vitro*.

Based on these assessments, benzene has been classified as a “Group 1” carcinogen to humans.

National Toxicology Program (2005):

Benzene is known to be a human carcinogen based on sufficient evidence in humans (NTP 2005). Case studies have reported leukemia (mostly acute myelogenous leukemia, also known as acute myeloid or myelocytic leukemia) in individuals exposed to benzene. The strongest epidemiological evidence that benzene causes cancer is from several cohort studies in various industries and geographical locations (IARC, 1982; IARC, 1987). Since benzene was reviewed for listing in the First Annual Report on Carcinogens and by the IARC, numerous epidemiological studies of benzene exposure have been published. Some studies found that the risk of leukemia increased with increasing benzene exposure; increased risk of death from leukemia was very high in the groups with the highest exposure (IPCS, 1993). In a review of 18 community-based and 16 industry-based studies of benzene exposure, Savitz and Andrews (1997) suggested that the evidence supported an association between benzene exposure and leukemia in general, rather than specifically with AML. Most studies found that benzene exposure increased the risks of total lymphatic and hematopoietic cancer (i.e., cancers of the lymphatic system and of organs and tissues involved in production of blood), total

leukemia, and specific histologic types of leukemia, including chronic lymphocytic leukemia, as well as acute myelogenous leukemia. Little evidence was found for an association between benzene exposure and multiple myeloma or non-Hodgkin's lymphoma.

The evidence in humans is supported by studies in experimental animals, demonstrating that benzene causes cancer at multiple tissue sites in rodents. Benzene was tested for carcinogenicity in mice and rats exposed by several routes, including oral administration, inhalation, injection, and dermal application. When administered orally, benzene caused Zymbal-gland carcinoma and oral-cavity tumors in rats of both sexes; skin carcinoma in male rats; Zymbal-gland carcinoma, malignant lymphoma, and lung tumors in mice of both sexes; harderian-gland adenoma and preputial-gland carcinoma in male mice; and ovarian tumors and mammary-gland carcinoma and carcinosarcoma in female mice (NTP 1986). When administered by inhalation, benzene caused tumors at many tissue sites in rats and a tendency towards lymphoid tumor induction in mice. Benzene administered by intraperitoneal injection caused benign lung tumors in male mice. No tumors were observed in mice administered benzene by subcutaneous injection or dermal application (IARC 1982, 1987). However, dermal application of benzene caused benign skin tumors in transgenic mice carrying the v-Ha-ras oncogene, which increases their susceptibility to carcinogens (Blanchard *et al.*, 1998, Spalding *et al.*, 1999, French and Saulnier 2000). Later studies reported that when administered benzene by gavage, heterozygous p53-deficient mice (with only one functional copy of the p53 tumor-suppressor gene) developed head and neck, thoracic cavity, and subcutaneous sarcomas (French *et al*. 2001, Hulla *et al*. 2001).

3.7 Epidemiology

A number of studies have attempted to develop risk assessments for benzene exposure based on the statistical modeling of occupational exposures, and some have been utilized by various jurisdictions to derive ambient air criteria for benzene (see Section 4). There is strong evidence from epidemiological studies that high level benzene exposures result in an increased risk of a specific type of leukemia – AML (Bergsagel *et al*., 1999; ATSDR, 2007). Marginal, non-significant increases have been observed for other cancers, including lung cancer and chronic myelogenous leukemia.

Along with AML, other types of cancer reported in some studies which may be caused by benzene exposure include acute lymphocytic leukemia, acute erythroleukemic leukemia, acute myelomonocytic leukemia, acute promyelocytic leukemia, acute undifferentiated leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, hairy cell leukemia, Hodgkin's lymphoma, lymphosarcoma, reticulum cell sarcoma, non-Hodgkin's lymphoma, and multiple myeloma (Wong and Raabe, 1995; Savitz and Andrews,1997; Mehlman, 2004). Specifically, in industrial workers, Mehlman (Mehlman, 2004) reported 11 cases of death by chronic myelogenous leukemia for a benzene exposure of 600 ppb-years – equivalent to 1920 $\mu g/m^3$-years, and 11 deaths due to multiple lymphomas for an exposure of 110 ppb-years (Rinsky *et al*., 1987) – equivalent to 352 $\mu g/m^3$-years. However, Bezabeth and colleagues have concluded that there is no specific evidence to support a causal relationship between

benzene exposure and multiple lymphomas (Bezabeth *et al*., 1996). Additionally, excessive cancer incidence has also been reported in workers exposed to benzene for lymphosarcoma, as well as cancers of the lung, liver, stomach, oesophagus, nasopharynx and intestine (Mehlman, 2004). However, epidemiological studies have also reported minimum or no correlation between non-AML leukemia and benzene exposure. (Lamm *et al*., 2005; Hartge and Smith 2007; Smith *et al*., 2007; Vineis *et al*., 2007; Steinmaus *et al*., 2008; Wang *et al*., 2009). While these non-conclusive results of other cancer endpoints may be cautiously used as weight-of-evidence, only the AML end-point can be considered for dose-response analysis in AAQC derivation.

Other cohort study analyses add further support and insight into benzene toxicity. Studied populations include both industrial workers and the general population, including children. For example, an Australian petroleum industry cohort study (Glass *et al*., 2003), showed that risk of leukemia increased with intensity of highest exposed jobs over 0.8 ppm (equivalent to approximately 2.59 mg/m^3). However, no evidence was found for a cumulative exposure threshold below which there was no risk (Glass *et al*., 2003). Occupational study on Lympho-hematopoietic cancer, between 1981 and 1999 (N = 79) showed that the risk of leukemia was increased at cumulative exposures above 2 ppm-years and with intensity of exposure of highest exposed job over 0.8 ppm. Risk increased with higher exposures; for the 13 case-sets with greater than 8 ppm-years cumulative exposure, the odds ratio was 11.3 (95% confidence interval = 2.85-45.1). No evidence was found of a threshold cumulative exposure below which there was no risk (Glass *et al*., 2003).

In another study, Lan *et al*. (2004) examined 250 workers exposed to benzene at a Chinese shoe factory at concentrations including less than 1 ppm (average 570 ± 240 ppb – equivalent to 1.85 ± 0.78 mg/m^3) in air (Table B of Appendix). Progenitor cell colony formation significantly declined even below 1 ppm (3.24 mg/m^3) of exposure, and was more sensitive to the effects of benzene than was the number of mature blood cells. Two genetic variants in key metabolizing enzymes – myeloperoxidase and NADPH: quinone oxidoreductase – increased susceptibility to benzene hematotoxicity. Significantly, hematotoxicity from exposure to benzene occurred at air levels at 1 ppm (3.24 mg/m^3) and below, and may be particularly evident among those who are genetically susceptible. In a study carried out by Qu *et al*. (2002), 130 Chinese workers exposed to benzene with 51 unexposed gender-matched controls showed depression of RBC, WBC and neutrophils. These effects were also observed at concentrations of approximately 250 ppb (0.8 mg/m^3). While these effects may not be considered as direct biomarkers of leukemia, they contribute to hematopoietic effects at low benzene doses. Additionally, a critical review on leukemia and low level benzene concentration (Brautbar and Wu, 2006) cites some of the data at ppb exposure levels, and includes the following:

1. Australian Institute of Petroleum (2000) found a 50% increase of leukemia in a 12.8 year follow-up, at an average benzene exposure of 200 ppb (~0.65 mg/m^3) (95% confidence)

2. An observable effect (i.e., incidence of leukemia) at as low as 16 ppb (~52 μg/m^3) was calculated by Brautbar and Wu (2006), by referring to the data analysis of both OSHA (2005) and Mehlman (2004)
3. Sajani *et al* (2005) examined the leukemia risk due to air pollution from benzene for a population living within an urban area of Italy. The leukemia risk at benzene levels of 2.16 ppb (~7 μg/m^3) for the year 2000 was compared to benzene levels 15.7 ppb (~51 μg/m^3) for the year 1988. The leukemia risk assessment was calculated based on cumulative benzene exposure and was in general agreement with Mehlman (2004) and OSHA (2005).

Together, these data along with other studies including, Creek *et al.* (1997); Lan *et al.* (2004); Qu *et al.* (2002); Nilsson *et al.* (1996); Glass *et al.* (2003); Parodi *et al.* (2003); Rappaport *et al.* (2002a; 2002b), support the fact that benzene is a hematotoxic and non-threshold leukemogenic agent (i.e., by incidence of leukemia) at low concentrations, ranging in the order of ppb to less than 10 ppm (Brautbar and Wu, 2006).

An additional study provides a correlation between leukemia and environmental exposure concentrations of benzene. Briefly, Parodi *et al*. (2003) investigated the risk of leukemia for a population exposed to benzene from a neighboring coke oven plant. The authors reported significantly increased risk for all lympho-hemopoietic cancer and leukemia in the male population (odds ratio = 1.7). Mean benzene concentration was 3.18 ppb (~10.3 μg/m^3), with a range of 0.86 to 6.33 ppb (2.8 to 20.5 μg/m^3). Other type of cancer clusters were predominant in this study, and thus do not add any specific support to any subtype occurrence. Nilsson *et al.* (1996) examined genotoxic effects in workers exposed to benzene at 100 ppb (~320 μg/m^3). This study compared single strand breaks (SSB) in DNA of leukocytes and urinary levels of oxidative DNA adduct biomarker 8-hydroxydeoxyguanosine (8OhdG) of 33 men occupationally exposed to benzene from gasoline, to a similar number of controls. The 8-hour time weighted average exposure to benzene was 130 ppb (~420 μg/m^3). Exposed workers had a significantly increased SSB over the control and significant increase in urinary 8OhdG. The authors concluded that genotoxic effects of benzene in humans occur at relatively low exposure levels of benzene of about 100 ppb (~320 μg/m^3). Brautbar and Wu's review (Brautbar and Wu, 2006) supports the belief that benzene is hematotoxic at ppb levels of exposure – specifically, evidence for a threshold in the carcinogenicity of benzene has not been identified. Furthermore, the human epidemiological studies, and *in vivo* and experimental animal studies, lend support to the recent Collegium Ramazzini appeal (Collegium, 2004) to consider the reduction of the occupational standard of 500 ppb (~1.62 mg/m^3) recommended by ACGIH in 1997.

Formation of micronucleus is an important mutagenic test in assessing the cell damage due to chemical insult. Increased micronucleus formation has been recorded in bone marrow cells and blood cells of mice exposed to benzene at high concentration (e.g. 50 ppm, 100 ppm) through inhalation (Valentine *et al*., 1996; Bauer *et al*., 2003; Recio *et al*., 2005; Wetmore *et al*., 2008).

Epidemiological studies on the frequency of formation of micronucleus due to benzene exposure have revealed suggestive results. Maffei *et al.* (2005) analyzed hematological

parameters and the frequency of formation of micronucleus in peripheral lymphocytes, along with various other hematological endpoints. The analysis was done in traffic policemen and the general public (Bologna, Italy), who were exposed to various concentrations of benzene. Increase in micronucleus formation in traffic policemen was thought to be associated with mean benzene concentrations greater than 20 $\mu g/m^3$, where the control subjects were only exposed to concentrations greater than 4 $\mu g/m^3$. However, the possibility of influence of chemical mixtures as a confounding factor is worth further discussion. Here, the authors state that most of the other environmental pollutants (e.g., NO_2, benzo(a)pyrene, etc.) are below any toxicological threshold. To support their claim, the authors recall data from their previous study at a mean benzene concentration of 14 $\mu g/m^3$, where no increase in micronucleus was observed (Violante *et al*., 2003), presumably with a similar exposure of other potentially confounding environmental pollutants. Moreover, the authors quote a Roman traffic police study at a mean benzene concentration of 9.5 $\mu g/m^3$ (Leopardi *et al*., 2003), where the results indicated that there was no observed increase in micronucleus formation. Based on these facts, the impact of environmental confounding factors (i.e., chemical interference) seems to have less effect on micronucleus formation than overall levels of benzene concentration. Overall, this discussion can only be a suggestive inference for the chemical mixture interaction as a confounding factor. It should be stated that these findings, however, do not support a possible dose-response analysis with a threshold approach. Rather, the Ministry views these data as very good qualitative weight-of-evidence for low dose effect, rather than an indication to quantitatively identify a toxicological point of departure.

Buthbumrung *et al*. (2008) have reported evidence of increased oxidative DNA damage in urban children from Bangkok schools (n = 109; mean benzene level 28.8 $\mu g/m^3$), when compared to rural school kids exposed to 14.6 $\mu g/m^3$ benzene. A significant increase of 8-hydroxy-deoxyguanosine (8-OHdG) in leukocytes and in urine was found in urban school children. However, only the GSTM1 genotype had a significant effect on the urinary muconic acid excretion, which can be attributed to polymorphic variation in the genotype. Buthbumrung et al. (2008) concludes that an increased health risk exists from traffic emissions that includes benzene.

A number of epidemiological studies have demonstrated the carcinogenicity of benzene in humans, and several regulatory agencies have considered these studies in setting their ambient air guidelines and standards. These studies include those involving the manufacture of Pliofilm (see Figure 3.6), a meta-analysis focusing on death due to cancer in petroleum industry workers (Wong and Raabe, 1995), and a meta-analysis of several dozen community-based and industry-based studies of benzene exposure (Savitz and Andrews, 1997).

1. Industrial Cohort Study - Pliofilm study

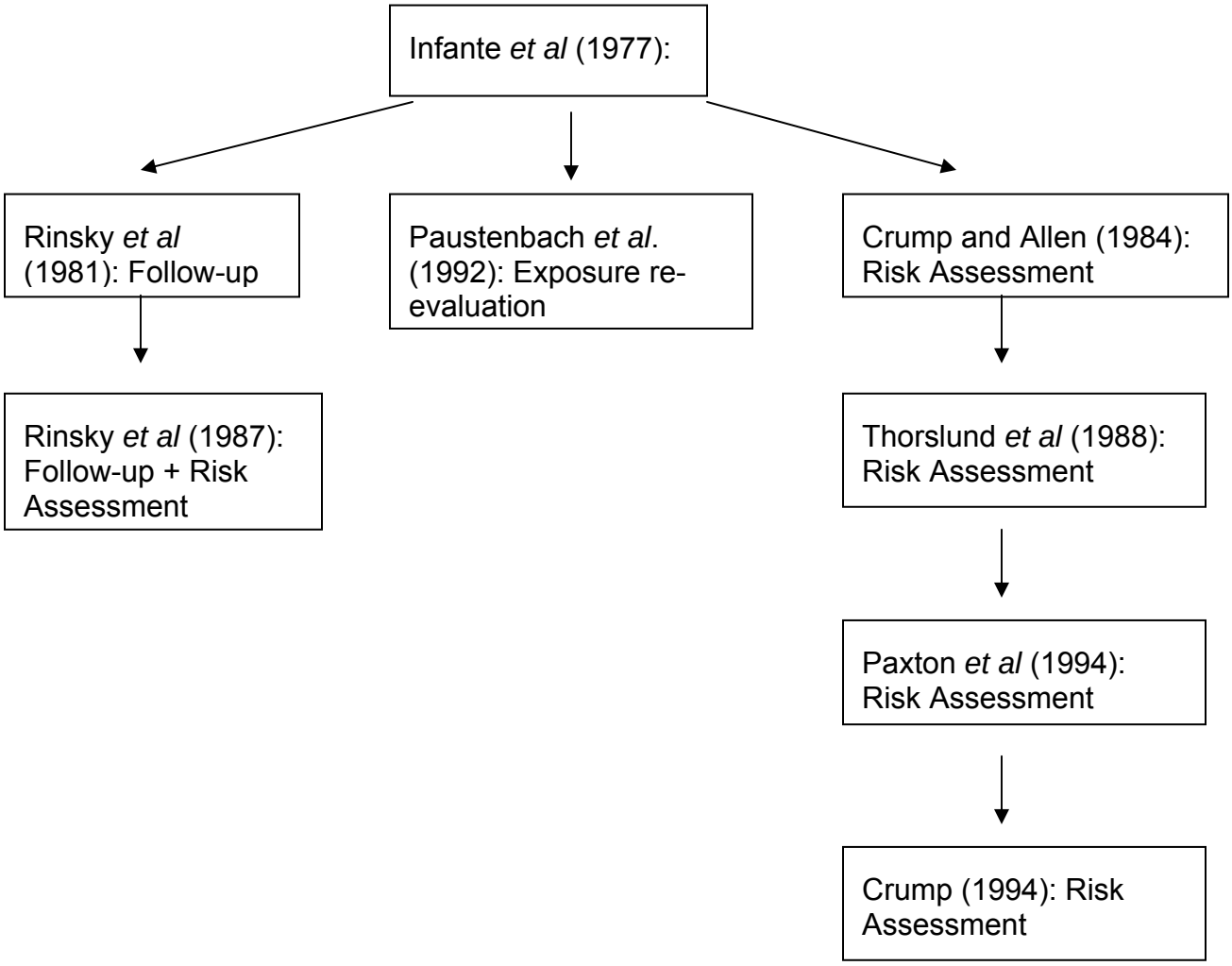
Cohort data were collected from workers involved in the manufacture of Pliofilm, a glossy membrane for industrial purposes (Infante *et al.,* 1977). The study involved two Pliofilm plants (St. Marys, Ohio; Akron, Ohio) which operated within or during 1937 to 1976. Cumulative exposure of workers was calculated based on the standard assumption that work/exposure is for 8h/day, 250 days/year, and 10 m^3 of air. From

this, four cumulative exposure groups were presented: 1) 0-45 (mean = 11) ppm-yr; 2) 45-400 (mean -151) ppm-yr; 3) 400-1000 (mean -602) ppm-yr; and, 4) 1000+ (mean -1341) ppm-yr – these exposure groups may also be expressed as exposure categories of 0-145 mg/m^3-yr, 145-1300 mg/m^3-yr, 1300-3200 mg/m^3-yr, and 3200+ mg/m^3-yr, respectively. The initial data collected by Infante and colleagues (Infante *et al*., 1977) was followed up by different groups, by tracking the incidence of leukemia and death. As well, re-evaluation of the exposure concentrations and scenarios were also conducted. Such follow-ups continued until 1987 (Paxton *et al*., 1994). Thus, this represents approximately 50 years of exposure and follow-up analysis.

Briefly, the Pliofilm analysis is based on 1717 white male workers employed during the years of plant operation. It has been suggested that this cohort is particularly suited for evaluating the health effects of benzene, due to both a relatively high exposure to benzene, and the absence of exposures to other toxic chemicals (Crump 1994). The background mortality data used were from 1973-1977, where U.S. white males and the Ohio leukemia rates during this period were closely approximated by U.S. rates (Crump 1994).

The initial follow-up describing incidence of cancer death was performed by Rinsky and colleagues (Rinsky *et al*., 1981; Rinsky *et al*., 1987). A subsequent exposure re-evaluation was carried out a number of years later by a different group (Paustenbach *et al*., 1992). Additionally, further analysis for dose-response relationships, and risk assessment using different linear and non-linear models have been developed by several groups – most notably by Crump and colleagues (Crump and Allen, 1984; Crump, 1994), Thorslund and colleagues (Thorslund *et al*., 1988), and Paxton and colleagues (Paxton *et al*., 1994). A schematic outlining the events involved in the analysis of the Pliofilm cohort is found in Figure 3-8. It should be noted, however, that there are additional contributors to the analysis of Pliofilm studies whose work has been incorporated and acknowledged within these cited publications.

Figure 3.8: The major flow of events involved in the analysis of Pliofilm cohort study



Definitions: Follow-up refers to the observation and recording of the additional cancer deaths after the original data collection by Infante et al., (1977); Exposure re-evaluation refers to the re-evaluation of the exposure matrix which includes dermal exposure, if any, short-term high level exposure, respirator use, bias of sampling devices used in earlier years, and a previously unaccounted for shutdown of the St. Mary plant during World war II, etc.; Risk assessment refers to the different dose-response models applied to the Pliofilm data, when follow-up and re-evaluation of exposure data became available.

Briefly, Crump and Allen (1984) applied statistical models to the original data generated by Infante *et al*. (1977), but failed to include the later follow-up studies. Here, they used both multiplicative risk and additive risk models, including cumulative and weighted exposures, and their analyses were based on grouped data (i.e., Poisson likelihood). An additive risk model assumes that benzene increases the background mortality rate by an additive amount through a mathematical function, whereas the multiplicative model assumes a multiplicative factor. The analysis of the Thorslund group (Thorslund *et al*., 1988) used the Crump exposure matrix with the follow-up information of the cohort, up to 1981. The only model used was additive risk model and the weighted

exposure matrix. The data generated from each individual were treated separately, rather than grouped (Paustenbach *et al*., 1992). A peer review committee recommended a number of suggestions on the Thorslund group analysis (Voytek and Thorslund, 1991), which lead to the development of a further risk analysis publication (Crump, 1994). This latter publication is further discussed below.

Pliofilm dose-response models (Crump, 1994)

Combining the Pliofilm exposure data, the follow-up studies until 1987, and an exposure matrix developed by Paustenbach (1992), Crump (1994) developed the benzene dose-response model. A summary of benzene exposure matrix and mortality due to AML of the Pliofilm study has been tabulated below (Table 3.2) (Crump, 1994).

Table 3-2. Observed and Expected Number of AMML (AML and Acute monocytic leukemia) deaths by cumulative exposure (Paustenbach et al., 1992 exposure matrix). Adapted from Crump, 1994.

Cumulative exposure, ppm-yr range (mean)	Person-years	AMML		
		OBS	EXP	RR
0-45 (11)	30,482	0-2	0.82	0.0-2.4
45-400 (151)	16,320	1	0.51	2.0
400-1000 (602)	4867	2	0.22	9.1
>1000 (1341)	915	5	0.6	82.8

OBS – number of observed death
EXP – number of expected death
RR – relative risk (OBS/EXP)

Briefly, cumulative benzene exposure and time weighted cumulative exposure were the exposure matrices used in this dose-response analysis. Additive (absolute) risk model and multiplicative (relative) risk model were applied to the exposure matrices. Both linear and non-linear versions of additive and multiplicative risk models were used. Both log-likelihood and likelihood (complete likelihood with each subject making an individual contribution) statistical test methods were used. In the process of using these matrices, a total of 94 dose-response models for leukemia (also includes AML) were presented and discussed in this analysis (Crump, 1994). Out of the different models used by Crump (1995), the linear extrapolation model (i.e. LMS – linear multistage model) (95% upper confidence interval) and the quadratic model (90% upper confidence interval) are the best fits for the Pliofilm data.

2. Epidemiological Meta-analysis – Wong and Raabe, 1995

Wong and Raabe (1995) carried out a meta-analysis of the published cohort studies involving benzene exposure and death due to cancer in 208,000 US and UK petroleum industry workers during 1937-1989. Here, cell-type-specific leukemia analysis was conducted on these individuals, where the four major cell-type-specific leukemia are: 1) AML; 2) Chronic Myelogenous Leukemia (CML); 3) Acute Lymphocytic Leukemia (ALL); and, 4) Chronic Lymphocytic Leukemia (CLL). A brief description of the cohort studies is presented in Table 3-3. The exposure concentration of benzene ranged from approximately 0.65 mg/m^3 to about 32 mg/m^3 (average of 0.7 mg/m^3) in the cohort studies used in this meta-analysis (Wong and Raabe, 1995). Comprehensive survey in the petrochemical industry by Raabe (1996) showed most measurements (8 hours and 15 minutes) to be less than 1 ppm (3.2 mg/m^3). In Raabe (1996), 60% of samples were less than 1 ppm (3.2 mg/m^3), 22% were 1-5 ppm (3.2 to 16 mg/m^3), and some were at even higher concentrations. It is worth noting that the authors examined the Pliofilm study in their meta-analyses, and through the use of linear extrapolation, the authors calculated the risk resulting in death due to cell-type-specific leukemia in industrial exposure concentrations (Wong and Raabe, 1995). The resulting analysis suggested that when cell-type-specific leukemia standardized mortality ratio (SMR) is considered, there is no difference in death ratios between the exposed industrial population mentioned in the cohorts and the general public in at least three of the major leukemic types, *when using linear extrapolation*. The authors' meta-SMR for different cell-type-specific leukemia for the above mentioned occupational group with 15 years of follow-up includes: 1) AML = 0.96; 2) CML = 0.82; 3) ALL = 1.22; and, 4) CLL = 0.79 (Table 3-4) (Wong and Raabe, 1995). Finally, the authors concluded that epidemiological data demonstrate that even with enough exposure levels over an extended period of time, benzene does not increase the risk of AML. The authors interpreted the data from more than 208,000 US and UK petroleum workers, suggesting that the benzene exposure meta-analysis showed that these workers were not at any increased risk for AML, as benzene exposure levels in the petroleum industry have been substantially lower than required to reach the observed threshold. The authors also suggest that based on the Pliofilm data (Rinsky *et al*., 1987; and others – see above), the initiation of nonlinearity (i.e., a threshold) may centre around 640 mg/m^3-ppm-years.

Table 3-3: US and UK Cohort studies identified in Wong and Raabe, 1995

Organization	Cohort location	Number of workers	Observation period	Total deaths
Amoco	10 refineries (US)	10763	1970 –1986	1405
Chevron	El Segundo (CA) refinery	4773	1950 –1986	1121
	Richmond (CA) refinery	8523	1950 -1986	2038
Chevron (Gulf)	Port Arthur (TX) refinery	17844	1937 – 1987	6799
Exxon	Baton Rouge (LA) refinery	9894	1970 – 1982	2000
	Baytown (TX) refinery	8722	1970 - 1982	1374
	Bayway (NJ) refinery	6860	1970 - 1982	1826
Mobil	Beaumont (TX) refinery	7119	1945 – 1987	2294
	Paulsboro (NJ) refinery	4855	1946 - 1987	1681
	Torrance (CA) refinery	1991	1959 - 1987	408
Shell	Two California refineries	4585	1973 – 1989	1051
	Deer park (TX) refinery	6831	1948 - 1983	1180
	Wood river (IL) refinery	9796	1940 - 1989	3627
Texaco	13 refineries (US)	19077	1947 – 1977	4024
	Production, pipelines (US)	11098	1946 - 1980	1886
American Petroleum Institute	Land-based terminal (US)	9026	1946 – 1989	2066
	Marine vessels (US)	9109	1946 - 1989	2695
Institute of Petroleum	Eight refineries (UK)	34569	1951 – 1989	10193
	Distribution centers (UK)	23306	1951 - 1989	8743
TOTAL	**Petroleum workers (US,UK)**	**208741**	**1937 – 1989**	**56411**

Table 3-4: Meta-analysis of cell–type specific leukemia in refinery workers (US, UK): 1) 1937-1989; and, 2) 1937 – 1989, with more than 15 years of follow-up. Source: Wong and Raabe, 1995. Definitions: AML, Acute Myelogenous Leukemia; CML, Chronic Myelogenous Leukemia; ALL, Acute Lymphocytic Leukemia; CLL, Chronic Lymphocytic Leukemia.

Cell type	Observed deaths	Expected deaths	Meta-SMR	95% confidence interval
(1) 1937-1989				
AML	78	84	0.93	0.73 – 1.16
CML	35	37	0.94	0.65 – 1.31
ALL	21	16	1.32	0.81 – 2.01
CLL	47	54	0.87	0.64 – 1.16
(2) 1937-1989 +> 15 years follow-up				
AML	138	143	0.96	0.81 – 1.14
CML	53	65	0.82	0.62 – 1.08
ALL	33	27	1.22	0.83 – 1.71
CLL	71	90	0.79	0.62 – 1.00

3. Review of epidemiological data – Savitz and Andrews, 1997

Savitz and Andrews (1997) reviewed 18 community-based and 16 industry-based studies of benzene exposure. The original intention of the authors was to conduct a quantitative meta-analysis of the aforementioned studies. However, the various discrepancies among studies (i.e., exposure assessments) generated complexicity, which made any quantitative analysis not feasible. Rather, the authors tracked the pattern of the leukemia sub-types by emphasizing the magnitude of relative risk estimates across studies. For example, some of the studies included exposure range of approximately 6.5 mg/m^3 to 65 mg/m^3, and greater. The endpoint was the occurrence of cancer incidence. Tables 3-5 and 3-6 refer to the individual cohort studies, and show the relative risk while considering benzene exposure and total lymphatic & hematopoietic cancers or total leukemia. Table 3-7 shows the calculated relative risk specific to different leukemia types. These epidemiological studies suggest that even though AML is an accepted endpoint for benzene exposure, other types of cancer, including other types of blood-related cancers, may also show increased incidence in exposed populations. This review of epidemiological studies grades the quality of the previously published data. Only the highest quality data, which was generated by systematic evaluation of job titles and environmental measurements, were assembled by the authors, and are reproduced in Tables 3-5, 3-6 and 3-7.

Table 3-5: Results of studies of benzene exposure and total lymphatic and hematopoietic cancers. Note that at least two industry-based and one community-based study had high relative risk due to benzene exposure when total lymphatic and hematopoietic cancer endpoints are considered totally. Source: Savitz and Andrews, 1997

Publication reference	Study setting	Reported cases	Relative risk
Vai (1989)	Community	15	13.3
Tsai (1983)	Industry	0	0.0
Decoufle (1983)	Industry	4	3.8
Travis (1994)	Industry	82	3.4

Table 3-6: Results of studies reporting on benzene and total leukemia. Note that when total leukemia is considered as the endpoint, there are many studies (both industry- and community-based) which had high relative risks (> 2.0). Source: Savitz and Andrew, 1997.

Publication reference	Study setting	Reported cases	Relative risk
Girard (1970)	Community	30	2.9
Ishimaru (1971)	Community	24	1.8
Tsai (1983)	Industry	0	0.0
Linos (1980)	Community	4	3.3
Rushton (1981)	Industry	18	2.3
Yin (1989)	Industry	25	5.7
Paxton (1994)	Industry	14	3.6

Table 3-7: Results of studies reporting on benzene and specific histologic types of leukemia

Publication reference	Study setting	Histologic type of Leukemia	Reported cases	Relative risk
Checkoway (1984)	Industry	Lymphocytic	4	2.5
Girard (1970)	Community	C. Lymphocytic	9	3.7
Linet (1987)	Community	C. Lymphocytic	31	0.9

Publication reference	Study setting	Histologic type of Leukemia	Reported cases	Relative risk
Bond (1986)	Industry	Myeloid	4	4.4
Ott (1989)	Industry	Lymphocytic	2	1.5
Girard (1970)	Community	Acute	17	3.0
Richardson (1992)	Community	Acute	22	1.3
Flodin (1986)	Community	Acute myeloid	0	0.0
Crane (1992)	Community	Acute myeloid	4	3.3
Wong (1995)	Industry	Acute myeloid	6	5.0

When specific histopathological leukemic types are considered, most of the types show increased relative risk either at the community or in the industrial study – the pattern is difficult to ascertain. The etiology of different types may vary and the genetic sensitivity of the sub-population assessed also may play a major role in developing a specific type of leukemia. The age factor also plays an important role in determining the cancer type. The authors acknowledge the occurrence of different leukemic endpoint due to benzene exposure. Source: Savitz and Andrew, 1997.

Finally, the National Cancer Institute has been conducting a comprehensive study of 74,828 benzene exposed workers employed from 1972 to 1987 in 672 factories in 12 cities in China (Yin *et al*., 1987, Yin *et al*., 1989, Dosemeci *et al*., 1994; Yin *et al*., 1994; Hayes *et al*., 1996; Yin *et al*., 1996; Hayes *et al*., 1997). Their findings suggested that workers exposed to benzene at average levels of less than 10 ppm (32 mg/m^3) are subject to a high risk of hematologic neoplasms (RR = 2.2, 95%). A combination of acute non-lymphatic leukemia and myelodysplastic syndromes produced a RR of 3.2 (95%). Additionally, the risk of non-Hodgkin's lymphoma was significantly elevated (RR = 4.2, 95%) for those with a sustained exposure to benzene that occurred 10 years prior to diagnosis.

Epidemiological Studies of Benzene Exposure – Strengths and Uncertainties for Regulatory Purposes

In addition to the toxicokinetic and toxicodynamic issues at environmental exposure concentrations, the epidemiological studies describing benzene exposures possess many strengths, as well as limitations. This is significant when using these data for extrapolation to environmental exposure concentrations, as is done in the establishment of benzene AAQC.

While quantitative dose-response may be difficult with the limited data available regarding benzene exposure to the general population, it is possible to use industrial worker exposure studies, as a way to generate relevant human exposure data. Many human studies, either large or small cohorts, have been assessed under varying

benzene exposure concentrations and conditions. The exposure range includes environmental exposure concentrations, which would be comparable to exposure of the general public, to higher exposures that would prevail only in occupational situations. Granted, the exposure concentration and duration measures alone may not be the sole determining factors of the apparent toxicity of benzene, as other interfering factors may play a role in the outcome of toxicity. To that end, the following uncertainties should be considered:

Uncertainty in exposure matrices

- Industrial exposure data are generally retrieved from the average exposure pattern that prevailed in that time of the day, rather than individual exposure data.

- Lack of properly controlled exposure scenarios. Often, control subjects are exposed to the environmental levels of the compound under study, and often it is difficult to assess their actual exposure. As well, the relative appropriateness of controls may lead to uncertainties (e.g., using a national average incidence of cancer death for a region-specific industrial study).

- The genetic variation among different groups of the population, as shown with regard to genetic polymorphisms in DNA repair enzymes, may lead to significant differences in disease susceptibility, and thus may be a major source of uncertainty.

- Occupationally-exposed workers are expected to be well-protected with personal equipment against any possible excessive exposure, which would not be a realistic assumption for the general population.

- It was not until 1970's that epidemiologic studies of individuals exposed to benzene were carried out and some of the earlier studies were crude (Wong and Raabe, 1995). Conversely, control technology may play a key role in exposure estimation during the latter part of study years, thus affecting exposure estimates.

Endpoint Perceptions and Complications

- Most of the epidemiological studies identified acute myeloid leukemia (AML) as the critical toxicological endpoint for toxicity assessment. The paradigm is that any leukemogenesis may end up in death. However, there are clinical pre-leukemogenic (especially for AML) events like hypoplasia and pancytopenia which prelude the leukemogenic events. Such clinical events may be under-reported in studies with leukemia as the clinical endpoint. Indeed, in all the human studies with benzene exposure considered for regulatory risk analysis, the endpoint is consistently death due to cancer, and not the occurrence of cancer, or any potential precancerous hematopoietic condition.

- Benzene was the first organic solvent linked to aplastic anemia. There is a dose-effect relationship between exposure to benzene and the incidence of development of cytopenia (Castro-Malaspina and O'Reilly, 2001). Chronic exposure of benzene has been associated with the development of aplastic anemia and leukemia. Benzene may generate metabolites that are directly toxic to stem cells. The course of the disease and the prognosis of pancytopenia during aplastic anemia are progressive and life threatening. Prognosis at diagnosis is closely correlated with the neutrophil count. Furthermore, the risk of infection, mainly bacterial and fungal and associated mortality is high in patients with super severe aplastic anemia (Castro-Malaspina and O'Reilly, 2001). From the above argument, it is clear that severe infection and death could happen in individuals exposed to benzene even before they reach the leukemic stage. Thus, this may reduce the number of reported deaths due leukemia, as the individuals may have died prior to reaching this terminal stage.
- Conversely, approximately 25% of patients with aplastic anemia are cured by immunosuppressive therapy. Bone marrow replacement is another predominant therapeutic approach (Castro-Malaspina and O'Reilly, 2001). By the end of the 1960s, replacement of the affected bone marrow transplant from a sibling donor was shown to be curative (Young and Barrett, 1995). In the 1970s, the observation of autologous marrow recovery after treatment with anti- lymphocyte sera suggested that non-replacement therapy could restore marrow function. Increasingly refined transplant regimens and intensified immunosuppressive therapy, as well as improved transfusion support and antimicrobials, have produced significant hematological improvement and long-term survival rate (Young and Barrett, 1995). The above information supports the treatable nature of aplastic anemia, which may otherwise eventually develop into AML. These procedures were developed from 1960's and were used in persons affected with aplastic anemia. Some of the subjects, though affected by benzene exposure (i.e., inflicted with aplastic anemia), were cured, because cures became available in the mid-1960s, and hence raises the possibility that the benzene/leukemia related deaths ultimately became lower than expected.

- As an additional complication, long-surviving patients with aplastic anemia who have been treated with immunosuppressive therapy, have an increased risk of developing AML (Socie *et al*. 2000; Kojima. *et al*., 2002) at a later stage.

Effect of chemical mixture interactions on the dose-response pattern

Interference from co-exposures to VOCs such as toluene, ethyl benzene and mixed xylenes would alter the metabolism of benzene (section 3.1.5), especially at occupational exposure levels. Since benzene metabolites are linked to the development of carcinogenic effects, such chemical interaction would likely affect the

overall dose-response pattern – which becomes important when extrapolating to low doses.

- Benzene exposure has been found to induce cytochrome P450 – most notably CYP2E1 – thus increasing the rate of toxic metabolite formation. In general, the oxidation of benzene by CYP2E1 has been demonstrated to be required for the expression of hematotoxicity and genotoxicity, and for the ultimate development of leukemia. Therefore, exposure to chemicals that stimulate the activity of this enzyme system *prior or concurrent to exposure to benzene* could increase the rate of benzene metabolism. As it is the benzene metabolites which are thought to be responsible for the toxicity of benzene, it remains a possibility that environmental exposures may lead to increased toxicity of benzene, due to this enzyme system activation (Daiker *et al*., 2000; Dennison *et al*., 2004; Mumtaz *et al*., 2004; Wharfe *et al*., 2004; Dennison *et al*., 2005a; Dennison *et al*., 2005b;; Yang and Andersen 2005). However, these effects may be in competition with enzyme-substrate binding effects.

- Chemical interactions are encountered in many of the epidemiological studies used to establish ambient air target levels for benzene (i.e. EU usage of petroleum worker cohort studies). Possible confounders include toluene, xylene, and ethyl benzene, among other VOCs. At high occupationally-relevant exposures, the chances of chemical mixture exposure having a significant effect on benzene metabolism are high.

- In contrast to the increased production of CYP2E1, most of the aforementioned interacting chemicals are competitive inhibitors of benzene metabolism (Haddad *et al*., 2001; Dennison *et al*., 2003). Hence at the interaction concentration range, it is likely that the metabolism of benzene is affected, which may lead to accumulation of parent compound (benzene) and its subsequent elimination, thus potentially lowering the production of the toxic metabolites.

Without a doubt, the data gleaned from epidemiological studies are valuable – however, special consideration must be taken in comparing the internal dosimetry at such high occupationally doses, and question whether similar internal dosimetry (i.e., chemical interactions) are to occur at lower, environmental exposure doses. As well, what effect these potential differences may have on extrapolating dose-response relationships down to ambient air levels needs to be considered. In this light, some of these issues are revealed upon performing PBTK model analysis (e.g., effect of chemical mixture exposure) on the available data (see Section 3.1.5).

Epidemiological studies: Selection criteria for AAQC development

Selection of Human exposure studies: Many animal toxicological and human epidemiological studies have been carried out regarding benzene exposure. With regard to animal models, there are no studies that clearly characterize a dose-response for AML due to prolonged exposure to benzene. Moreover, toxicokinetic understanding

(Section 3.1) indicates the possibility of human/animal differences in the rate of metabolism of benzene. Under such circumstances, human epidemiological studies with clear dose-response data for an AML endpoint are the preferred choice for further analysis. Nonetheless, animal studies remain vital in the mechanistic understanding, which is important during low dose extrapolation of cancer risk.

Pliofilm Study: This is a well characterized study with thorough estimation of exposure analysis and good follow up of AML mortality assessment. Chemical interference from other volatile organic compounds is not expected since benzene was the only solvent used in the Pliofilm industry. Pliofilm study has been used for dose-response analysis by different jurisdictions and satisfies most of the Hill Criteria– an index of cause-effect association (Yardley-Jones and Gray, 2001; Descatha *et al*., 2005).

Wong and Raabe: This meta-analysis involves studies from petrochemical industries along with Pliofilm study for analyzing the dose-response extrapolation to low benzene exposures. This is one of the largest meta-analysis that summarizes results from various petrochemical industries, and also includes hemapoietic cancers other than AML.

Savitz and Andrew: This meta-analysis includes references to both industrial and general human population exposure data and multiple cancer endpoints including AML. This study, in part, notes the occurrence of hemapoietic cancers, including AML, rather than just mortality.

Other epidemiological studies: Other epidemiological studies summarized in this document were used in a qualitative and/or weight-of-evidence manner for the dose-response analysis at exposure concentrations below those assessed at occupational levels. Low dose epidemiological studies not only examine the incidence of hematopoietic cancers (including AML), but also the molecular events or the metabolism component of benzene toxicology. Despite some contradictions, most of the epidemiological studies provide prudent scientific facts and support the low dose risk of benzene; however the use of such data for dose-response quantification remains questionable, due to the influence of multivariant environmental factors.

3.8 Low dose Considerations

A number of jurisdictions set their health-protective benzene guideline values by extrapolation from occupational exposure concentrations down to environmental exposure concentrations. The agencies do this with varying degree of confidence (discussed in Section 4), and acknowledge the significant shortcomings of extrapolating over such large magnitudes of exposure. Thus, it is worth noting some of the toxicologically-relevant issues about low dose exposures, which may not be necessarily observed at occupationally-relevant exposure levels.

A recent paper highlights evidence of the formation of the toxic metabolites of benzene, and the subsequent formation of DNA and protein adducts, at exposure equivalent doses as low as 1.6 $\mu g/m^3$ – i.e., an ambient air concentration. Here, macromolecular binding of benzene metabolites was dose-dependent at low doses of benzene exposure

(Turteltaub and Mani, 2003). Dose-response for the metabolites of benzene (muconic acid, hydroquinone and catechol) were linear in mice liver samples for a range of 5 ng/kg bw to 5000 ng/kg bw, following intraperitoneal injection (Turteltaub and Mani, 2003). These doses correspond to 1.6 µg/m^3 to 1600 µg/m^3 equivalent in 30 g mice. Turteltaub and Mani (2003) hypothesize that the differences in the capacity of various species of mice and rats to metabolize benzene affect macromolecular adduct formation, and that the amount of macromolecular damage is related to benzene's ability to cause cancer and other blood disorders. The estimates of linear regression of log metabolite response on log benzene dose for mice liver after 1 hour of benzene exposure (IP) showed a most effective dose-response for muconic acid with a slope estimate of 1.08 ± 0.08 (R^2 of 0.96) and for hydroquinone the slope estimate was 0.71 ± 0.07 (R^2 of 0.94). Both DNA and protein adduct formed by benzene metabolites also showed dose-response pattern in liver and bone marrow of mice exposed to benzene IP at the above dose range. Area-under-curves (AUCs) of protein and DNA adducts in bone marrow are consistent with the capacity to bioactivate benzene and with benzene's tumorigenecity among mice and rats (Huff *et al*., 1988; Huff *et al*., 1989). Thus, while species differences must be acknowledged when extrapolating from rodent to humans, the above experimental evidence shows that rodents metabolize benzene effectively at all doses, including those relevant to human exposure, and that DNA adduct level reflects the ability of rodents to potentially metabolize benzene to toxic metabolites.

There is evidence that low-dose exposures to benzene favoured the production of hydroquinone and muconic acid, which are considered to be more contributory in the process of carcinogenesis due to benzene exposure. In fact, in most species that have been studied, a greater portion of benzene is converted to hydroquinone and muconaldehyde when the dose is low. This statement is consistent with the Turteltaub and Mani (2003) experiments, and as well appears to be true with humans, where both hydroquinone and muconic acid have been detected in the urine of exposed workers (Rothman *et al*., 1998) (see Section 3.1.3). Briefly, in the key study, dose-related production of the four major metabolites (hydroquinone, muconic acid, phenol and catechol) and total metabolites (µM/L/ppm) declined between 2.5- and 26-fold as group median benzene exposures increased between 86 µg/m^3 and 50 mg/m^3 (Figure 3-9) (Kim *et al*., 2006). In the lower range (from 86 to 876 µg/m^3), production of the "less toxic" metabolites (catechol and phenol) increased by 16- and 4.4-fold, respectively, while there was only marginal increases in the production of hydroquinone and muconic acid (44% and 36%, respectively). Thus, in this study there seems to be an increase in the production of all metabolites relative to level of benzene (i.e., approaching linearity) as the air benzene concentration is lowered – including the more 'toxic' metabolites, albeit at a lower rate than the less toxic metabolites (Figure 3-9). Specifically, of all the benzene metabolites, 1,4-benzoquinone (derived from hydroquinone) has most often been linked to the spectrum of toxic effects (Smith, 1996; Snyder, 2000a; Snyder, 2000b; Inayat-Hussain and Ross, 2005). These studies are consistent with the phenomenon observed where lower amounts of metabolites were observed in the more highly occupationally exposed (Sabourin *et al*., 1988a; Sabourin *et al*., 1988b; Sabourin *et al*., 1989).

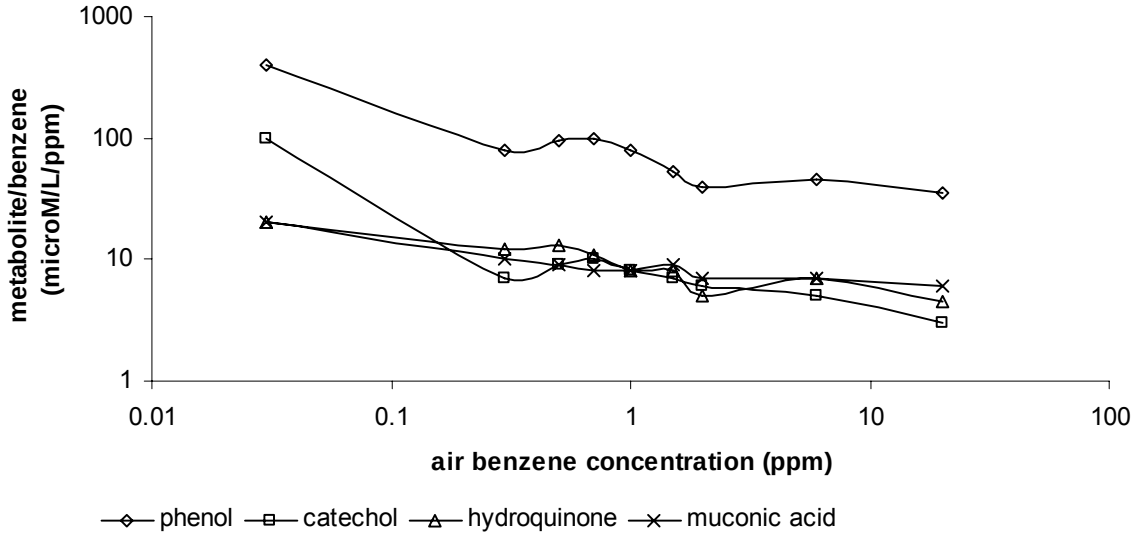


Figure 3.9: Metabolite concentration of benzene produced due to varying exposure concentration of benzene (Source: Kim et al., 2006)

Although to a lesser extent than muconic acid and hydroquinone, phenol and catechol are also considered to affect the process of benzene-induced non-hematological carcinogenesis. For example, the primary oxidation of benzene to phenol and subsequently to catechol and catechol quinone, leads to quinone reacting with DNA by 1,4-Michael addition to form depurinating adducts (Cavalieri and Rogan, 2004; Cavalieri and Rogan, 2006).

The dose-response underlying the above observation (Figure 3.9) points to saturation of CYP2E1 as a critical metabolic consequence of high exposure to benzene in humans. For example, cysteinyl adducts of serum albumin was used to investigate the production of benzene oxide and 1,4- benzoquinone in workers exposed to benzene (Rappaport *et al*., 2005). In occupational studies where benzene levels can be very high, one would anticipate that transient days of exposure greater than about 50 ppm (160 mg/m^3) would have diminished impact upon the leukemic risk, since benzene metabolism is substantially saturated in this region (Hayes *et al*., 1997; Glass *et al*., 2003; Rappaport *et al*., 2005). Thus, the biologically effective dose of benzene oxide and 1,4 benzoquinone may be proportionally greater in persons exposed to low rather than high levels of benzene (Rappaport *et al*., 2002a; Rappaport *et al*., 2005).

In contrast to the linear low dose extrapolation of the formation of metabolites, a recent study Rappaport *et al*., (2009) provided evidence of increased efficiency of metabolism at low doses. Total urinary metabolites of benzene in 263 Chinese females were analyzed from an exposure range of over 1 ppb (3.2 µg/m^3) to less than 100 ppm (320 mg/m^3). Applying Michaelis-Menton like curve fitting models with regression analysis lead to the observation that better model fit was possible with biphasic metabolic constants (V_{max} and K_m). The first curve acting above 1 ppm is considered as the low affinity pathway. The second high affinity pathway acts below 1 ppm to as low as 1 ppb (unknown pathway). The exposure specific metabolite level predicted by two pathway

model (biphasic metabolic constants) resulted in total metabolite concentration of 184 µM/ppm, whereas, single pathway model resulted in a total metabolite concentration of 68.6 µM/ppm. Considering the above result, a combination of two pathways (proposed as low and high affinity pathways) depending on the exposure pattern, a three-fold increase in the total amount of metabolites formed per unit benzene exposure was observed when compared with the single pathway model. Hence, there is a potential for benzene exposure at low concentration to produce more molar concentration of metabolites per unit benzene exposure. The authors further conclude that the true leukemia risk could be greater by a factor of three at the environmental exposure concentration than that was originally predicted in general population. This data published by Rappaport *et al*., (2009) adds further support to Kim *et al*. (2006). This biphasic enzyme kinetic pathway is considered as a weight-of-evidence for a possible supralinear risk of effect at low doses of benzene exposure.

Additional supportive evidence of toxicity at environmental exposure concentrations of benzene comes from epigenetic studies of aberrant DNA methylation patterns, including global hypomethylation, gene-specific hyper/hypomethylation and loss of genetic imprinting are common in AML and other cancer tissues (Greiner *et al*., 2000; Galm *et al*., 2006; Bollati *et al*., 2007). The human benzene exposure study of Bollati *et al*. (2007) linked altered DNA methylation, reproducing the aberrant epigenetic patterns found in malignant cells, to low level benzene exposure. Loss of genetic imprinting (e.g., altered methylation, as part of the AML disease process) was observed in humans exposed to benzene levels as low as 28 to 276 µg/m^3. It should be noted, however, that the authors commented that they could not exclude the possibility of inferences from other environmental pollutant including particulate air matter, PAH, CO, SO_2, NO_2, toluene or xylene in the observed changes. This statement has since been reconfirmed (Baccarelli *et al*., 2009)

Evidence from extensive chromosomal studies have led scientists to conclude that exposure to benzene-induced chromosomal aberrations may be a predictor of future leukemic risk (Forni 1996; and Au *et al*., 2002). Indeed, the induction of health problems by chemicals such as benzene is frequently mediated by the induction of persistent effects in cellular macromolecules (Loeb 2001). For example, the induction of cytotoxicity and DNA strand breaks by benzene and its metabolites was demonstrated in Chinese hamster ovary cells exposed to benzene and its metabolites (Sze *et al*., 1996). The order of the cytotoxicity exhibited by these chemicals are as follows; 1,4-benzoquinone > hydroquinone > catechol > tt-muconic acid (tt-MA) > 1,2,4-benzenetriol > S-phenylmercapturic acid (S-PMA) > phenol > benzene, thus relating the toxicity of the metabolites of benzene to the molecular toxicity rather than the parent compound itself. Increased recombination which may lead to adverse genetic change was observed in Chinese hamster ovary cells exposed to phenol, catechol or benzoquinone (Winn, 2003). Qu *et al*. (2000) reported that urine tt-MA and S-PMA are sensitive biomarkers for exposure of benzene and has been demonstrated at levels of 320 - 3200 µg/m^3. Waidyanatha *et al*. (2001) examined urine benzene metabolites in benzene-exposed workers and concluded that urinary metabolites are specific biomarkers of exposure at less than 3.2 mg/m^3 exposure. Benzene DNA adducts have been found in animals exposed to benzene 10 years ago (Li and Wang, 1996).

Yeowell-O'Connell *et al*. (2001) reported that benzene oxide-albumin adduct and 1, 4-benzoquinone albumin adducts are 2.4 times higher in benzene exposed workers. This relationship is apparently linear at lower benzene exposures but not at high exposure levels. Lymphocytic chromosomal aberration has also been reported (Gao *et al*., 2002). Many cytogenetic investigations have documented that occupational exposure to benzene caused a dose- and time- dependent increase of chromosome aberrations (Carere *et al*., 1998; Smith *et al.*, 1998; Tompa *et al*., 1994; Zhang *et al*., 1999; Smith and Rothman 2000; Au *et al*., 2002). Long-term follow-up of benzene exposed subjects revealed that chromosomal aberrations appear to be predictive of cancer risk (Forni, 1996). Benzene is also reported to induce aneuploidy in lymphocytes of exposed workers (Zhang *et al*., 1999). Clonal evolution of damaged cells in AML patients frequently showed loss of all or parts of chromosomes 5 and/or 7 (Forni, 1971; Le Beau, 1990).

3.9 Ecotoxicological Effects

Acute and chronic benzene toxicity is observed in different species from a number of trophic levels, from bacteria and protozoa through to fish and amphibians in the aquatic environment. The most sensitive freshwater invertebrates include *Ischnura elegans,* which has a 48-hour LC_{50} of 10 mg/L (Sloof, 1983). The most sensitive fish species tested were salmonids, including rainbow trout, *Oncorhynchus mykiss,* with a 96-hour LC50 of 5.3 mg/L for juveniles (DeGraeve *et al.,* 1982), and Coho salmon, *Oncorhynchus kisutch,* with a 96-hour LC_{50} of 9 mg/L for fry (Moles *et al.,* 1979). Benzene is toxic to a range of insects following topical or inhalation exposure; lethal effects were reported following exposure to air concentrations of 10 000 to 210 000 mg/m^3 (Miller *et al.,* 1976). Acute effects of benzene on terrestrial plants have been reported at atmospheric concentrations greater than 10 000 mg/m^3 (Miller *et al.,* 1976).

The most sensitive response reported for exposure to benzene in an aquatic organism is a 9-day LC_{50} of 3.7 mg/L for the leopard frog (Black *et al.,* 1982). This value can be multiplied by a factor of 0.05 to convert the LC_{50} to a chronic no-observed-effect concentration (NOEC) for a non-persistent, non- bioaccumulative substance and to account for differences in species sensitivity and extrapolation from laboratory to field conditions. This yields an estimated effects threshold of 185 µg/L for long-term exposure. The highest reported mean concentration of benzene in ambient freshwater in Canada is 2 µg/L; this is 1850 times lower than the LC_{50} for the leopard frog and 93 times lower than the estimated effects threshold. Therefore, benzene is not considered to be "toxic" to freshwater organisms exposed to ambient surface water.

Benzene is not considered to be "toxic" as defined under Paragraph 11(a) of CEPA (CEPA 1993).

Only limited work has been published in the 21st centaury in terms of effect of benzene to the ecosystem. *Pseudomonas putida* contains the TOL plasmid, responsible for the degradation of benzene and its derivatives. This bacterial species is omni-present and can degrade benzene to innocuous substances (Berno *et al*., 2004). Increase in micronuclei of the erythrocyte of fish at 10 ppm concentration reveals the genotoxic nature of benzene in aquatic fauna (Al-Sabti, 2000).

Benzene does not bioconcentrate in aquatic biota to a significant degree. Relatively low bioconcentration factors (BCFs) have been reported for aquatic bacteria, algae, macrophytes, and fish. The highest reported value was for *Daphnia pulex*, with a BCF of 225 (log BCF of 2.35) (Trucco *et al*., 1983). Even though aquatic toxicity has been cited, the solubility of benzene from air to water is very limited and hence the contribution from benzene aerial deposition and accumulation in water bodies are not expected. Plants and animals of lower phylogenic nature than mammals are not expected to be affected due to aerial exposure or inhalation of benzene at concentrations lower that could affect mammals.

4.0 Jurisdictional Review – Guidelines and their Derivations

Numerous inhalation unit risk factors and air quality criteria have been developed for benzene. The discussion below is separated into guidelines derived for carcinogenic or non-carcinogenic endpoints. The discussion will focus on the inhalation-based guideline values, unit risks, and the agencies' rationales in setting such values.

While it should be mentioned that a number of agencies have detailed rationale supporting their guideline values developed for the other routes of exposure (e.g., oral, drinking water), we will not be highlighting these values to the same extent in this section. As discussed in the proceeding section, however, there is minimal variation in the toxicokinetics of benzene as a result of different route of exposure, and no difference in the toxicodynamics. It should be noted that the experimental and epidemiological studies supporting the jurisdictional guidelines have been discussed in greater detail in Section 3.

4.1 Carcinogenic Effects

All regulatory agencies have considered leukemia risk as the key health risk. Specifically, based on epidemiological studies and toxicological studies in animals, the development of specific types of leukemia (e.g., AML) following inhalation exposure appears to be the critical adverse effect for humans.

Although the empirical support for this comes only from occupational inhalation exposure, there is the presumption that there would be a similar hazard associated with other routes of exposure in humans. In laboratory animals, carcinogenic potential has been demonstrated in both oral and inhalation studies. There are many reports of chromosomal damage in humans indicating benzene's genotoxic potential. This might lead to a default assumption that the dose-response of any benzene-induced cancer is unlikely to exhibit a dose threshold. In fact, the potency of benzene at *lower environmental exposure concentrations may have a higher potency due to enzymatic kinetics, as compared to the potency at occupational exposure levels* – this concept will be further discussed as an underlying consideration in the paths forward in Section 5.

4.1.1 Health Canada (1996)

Benzene has been classified as a Group I carcinogen (carcinogenic to humans), based on the evidence of carcinogenicity in humans and experimental animals (HC, 1996). This classification scheme was developed by the Bureau of Chemical Hazards for use in the derivation of the "Guidelines for Canadian Drinking Water Quality" (Health and Welfare Canada, 1986). This designation implies that carcinogenicity is potentially the most sensitive endpoint for the assessment of the toxicity to humans for benzene, under CEPA (CEPA, 1993). In numerous case studies, and in the majority of epidemiological studies conducted until 1996, associations between leukemia and exposure to benzene in occupationally exposed populations have been observed. In addition, Health Canada notes there was a clear exposure-response relationship in the population for which exposure has been the most extensively characterized (Rinsky *et al.,* 1987). Benzene

has also been consistently clastogenic in occupationally exposed populations, inducing both structural and numerical chromosomal aberrations in human lymphocytes (CEPA, 1993). Benzene has also been carcinogenic in two species of experimental animals, inducing a wide variety of tumours following inhalation and ingestion. Available data on the mechanisms of action of benzene also indicate that induction of leukemia by this compound is biologically plausible.

Health Canada proposed a tumourigenic concentration value (TC_{05}) of 15 mg/m^3, based on the cohort of Pliofilm workers described by Rinsky *et al*. (1987). The derivation of quantitative estimate of carcinogenic potency was based on AML. A linear quadratic absolute risk model was used to derive the TC_{05}. Health Canada notes that to provide a practical application of tumourigenic concentrations, the TC_{05} can be divided by a suitable margin of safety (i.e., 5000 to 50,000) to afford similar protection to that associated with the range for low dose risk estimates generally considered by various agencies to be "essentially negligible" (i.e., 10^{-5} to 10^{-6}, respectively).

4.1.2 US EPA – IRIS (2000)

Under the revised Carcinogen Risk Assessment Guidelines (US EPA, 1996), benzene is characterized as a known human carcinogen for all routes of exposure based upon convincing human evidence as well as supporting evidence from animal studies (reviewed in US EPA, 1979; US EPA, 1985; US EPA, 1998; ATSDR, 2007).

The US EPA noted that epidemiologic studies and case studies provide clear evidence of a causal association between exposure to benzene and acute non-lymphocytic leukemia (ANLL) and also suggest evidence for chronic non-lymphocytic leukemia (CNLL) and chronic lymphocytic leukemia (CLL). Other neoplastic conditions that are associated with an increased risk in humans are hematologic neoplasms, blood disorders such as preleukemia and aplastic anemia, Hodgkin's lymphoma, and myelodysplastic syndrome (MDS). The experimental animal data also indicate that exposure to benzene increases the risk of cancer in multiple species at multiple organ sites (hematopoietic, oral and nasal, liver, forestomach, preputial gland, lung, ovary, and mammary gland). These responses are likely due to interactions of the metabolites of benzene with DNA (Ross, 1996; Latriano *et al*., 1986). Evidence supports the viewpoint that there are likely multiple mechanistic pathways leading to cancer and, in particular, to leukemogenesis from exposure to benzene (Smith, 1996).

The supporting evidence for the carcinogenic effects of exposure to benzene comes from the understanding of the metabolism and mode-of-action as described by US EPA in 1998, using data from a number of studies (Medinsky *et al*., 1989; Stephens *et al*., 1994; Lee *et al*., 1996; Valentine *et al*., 1996; Rothman, 1997). Although there is a scientific consensus that metabolism of benzene is required for resultant toxicity and carcinogenic response, the role of a single metabolite or group of metabolites of benzene in producing these adverse effects needs more research data to better define the sequelae of pathogenesis following exposure to benzene and its metabolites. Evidence during the 1998 US EPA review indicated that benzene-induced myelotoxicity and genotoxicity result from a synergistic combination of phenol with hydroquinone, muconaldehyde, or catechol.

The study of Pliofilm workers (Rinsky *et al*., 1981; Rinsky *et al*., 1987) at 3 facilities in Ohio was determined to provide the best published data set to quantitatively evaluate human cancer risks from exposure to benzene. The extensive analyses by Crump (Crump, 1992; Crump, 1994) of the Pliofilm data (Rinsky *et al*., 1981; Rinsky *et al*., 1987) have been used in the more recent EPA evaluation (US EPA, 2000) as the basis for its estimates of the risk of leukaemia arising from the inhalation of benzene. The first Pliofilm study evaluated a cohort of 1165 male workers who had been employed between 1940 and 1965, and followed through 1981 (Rinsky *et al*., 1981). The second study included an additional 6.5 years of follow-up (Rinsky *et al*., 1987), with no additional death due to AML observed. By the end of 1981, 9 leukemia cases were observed versus 2.66 expected in this cohort. Relative risks were found to increase with cumulative exposure. Paustenbach *et al*. (1992) and Crump and Allen (1984) used various assumptions to estimate personal exposures prior to 1950, when exposures were most intense. Exposure estimates by Rinsky *et al*. (1981, 1987) were generally the lowest of the 3 sets, resulting in higher cancer unit risk estimates. Crump (Crump, 1992; Crump, 1994) reported 94 dose-response based unit risk calculations using different disease endpoints, different models (additive or multiplicative), linear/nonlinear exposure-response relationships, and different exposure measurements. Crump, assessing the influence of different mathematical models (additive or multiplicative), linear or non-linear dose-responses, and differing estimates of worker exposure (Crump and Allen, 1984; Paustenbach *et al*, 1992), presented almost 100 estimates of the cancer risk posed by an concentration of 3200 $\mu g/m^3$ (1 ppm) of benzene in air (i.e., in the industrial setting). These unit risk values ranged between 8.6×10^{-5} and 2.5×10^{-2} for all the models including both linear and non-linear patterns. These correspond to a range of 2.2×10^{-6} to 7.8×10^{-6} per $\mu g/m^3$ benzene if linear extrapolation is carried out. For all models (i.e., linear and non-linear), the specific benzene concentration at this unit risk ranged from 37 $\mu g/m^3$ to 0.13 $\mu g/m^3$ for 1 in a 10^6 population. This represents a range of approximately 285-fold. However, Crump (1994) indicates that the Paustenbach et al. (1992) exposure matrix was based on a much more detailed investigation than that by Crump and Allen (1984) and is likely to provide a better representation of exposures in the cohort. Moreover, Paustenbach et al. (1992) study also includes the follow-up of mortality, recorded in the Rinsky *et al* (1987) study.

The US EPA pointed out that the risk estimate would be in the lower region of this range if a sublinear exposure model was the most plausible, and in the higher region if the dose-response curve were supralinear at low exposures (and noted that the study of Hayes *et al.* (1997) of genetic abnormalities in humans offered some support for supralinearity). Further, US EPA considers both pros and cons of the linear extrapolation method as briefed in the following table for low dose extrapolation consideration.

Arguments for and against benzene-induced leukemia linearity at low doses (U.S EPA, 1998)

Pro	Con
Micronucleus assay is relatively insensitive and may not show effects at low doses.	Micronucleus induction by benzene and its metabolites in mouse bone marrow and in human cells in vitro is nonlinear.
Induction in aneuploidy of other chromosomes (e.g., 7) occurs at lower doses and the effect of benzene on hyperdiploidy of chromosomes 7, 8, and 9 shows a significant linear trend.	The induction of aneuploidy of chromosome 9 is nonlinear and is significant only at high levels of exposure (>31 ppm in air)
Data obtained using accelerator mass spectrometry shows that the formation of DNA adducts in mouse bone marrow is linear to very low doses.	DNA adduct formation is observed by P^{32}-post-labelling only at high doses.
Errors during repair may cause point mutations.	Oxidative DNA damage may contribute to benzene genotoxicity but has a high rate of repair.
Hematotoxicity may increase risk of malignancy but has not been shown to be a prerequisite.	Hematotoxicity is required for leukemia induction, and this will have a threshold.
There is a high background of exposure to benzene and its metabolites. Additional environmental exposure will simply add to this and be linear. There are also numerous mechanisms of aneuploidy induction, and aneuploidy is not the only mechanism of suppressor gene loss and oncogeny activation.	If aneuploidy is critical, then leukemia induction is likely to have a threshold. (Numerous molecules of benzene metabolites will be required to disrupt microtubules.

Pro	Con
There is a high background exposure to benzene and its metabolites, so additional exposure could escape defences.	The cells in the bone marrow have numerous defence mechanisms.

The above table qualitatively weighs the pros and cons of the mode-of-action understanding during the consideration of the low dose extrapolation model as proposed by US EPA in their 1998 analysis. Overall, the US EPA considered that there was currently no convincing scientific basis for the choice of any particular extrapolation model, and therefore believed that the use of a linear model as a default approach was appropriate. When a linear model was used, the estimates of unit risk narrowed to a range between 7.1×10^{-3} and 2.5×10^{-2} at 1 ppm (or 2.2×10^{-6} and 7.8×10^{-6} per $\mu g/m^3$ benzene) (US EPA, 2000). This corresponds to a risk specific annual concentration of 0.13 $\mu g/m^3$ to 0.45 $\mu g/m^3$ at a 10^{-6} risk level.

The IRIS oral cancer unit risk was extrapolated from the inhalation unit risk estimates. The resulting oral slope factor was expressed as a range of 1.5×10^{-2} per (mg/kg-day) to 5.5×10^{-2} per (mg/kg-day). From this, a drinking water guideline was determined. Here, the US EPA set the maximum contaminant level (MCL) for benzene as 5 µg/L for drinking water under the Safe Drinking Water Act.

4.1.3 European Union (1998)

The European Union formed an ad hoc expert working group on benzene (i.e., the EU panel) to develop recommendations for benzene limit values for its member countries. For the purpose of guideline derivation for benzene, essentially two epidemiological studies were identified to be of major importance: the Pliofilm cohort (analysed in Crump, 1994), and a large meta-analysis (Wong and Raabe, 1995). The EU group identified that the linear extrapolation of the Pliofilm cohort study did not match with the results of the 1995 Wong and Raabe meta-analysis, and built their risk analysis based on this observation. Specifically, the EU identified that the data from the comprehensive analysis of the petroleum industry meta-analysis (Wong and Raabe, 1995; Rushton and Romaniuk, 1997), which included more than 208,000 petroleum workers with an estimated average exposure of 0.7 mg/m^3, showed 148 cases of AML, whereas 155 were to be expected, considering standardized mortality rates. Thus, the EU panel concluded that based on these epidemiological data, a sublinear association between exposure and response could occur and that the linear extrapolation of occupational risk estimates based on high exposure conditions (several hundred mg/m^3) is likely to produce a substantial over estimation of the leukemia risk at lower exposure.

Due, in part, to such shortcomings, the EU panel concluded that although it was not possible on the then-current available evidence to give a precise estimate of the risk associated with benzene, it was possible to define a range within which that risk was

likely to lie. The procedure followed by the WHO working group was considered by the EU to result in the highest plausible estimate of risk – an excess lifetime risk of leukemia at an air concentration of 1 µg/m^3 of 6 x 10^{-6}. The lowest unit risk which the group felt was likely to be plausible was in the order of 5 x 10^{-8} (i.e., a back calculation from the selected value of 20 µg/m^3, corresponding to a 10^{-6} risk level) – an estimate which is consistent with the Netherlands Health Council analysis of the Wong and Raabe meta-analysis (see Section 4.1.4).

To elaborate, it can be noted that on the basis of the Wong and Raabe data, the Dutch Health Council calculated that continuous lifelong exposure to 35 µg/m^3 was not associated with an increased risk of leukemia (RIVM). Based on this figure, which is approximately three times greater than the limit proposed in the previous decade (i.e., 12 µg/m^3), the Dutch Health Council noted that this latter limit now corresponds to an "accepted" (extra) risk of one case of acute non-lymphatic leukemia per million mortalities, namely 12 µg of benzene per m^3 of outdoor air. The 20 µg/m^3 lowest plausible risk level selected by the EU expert group thus lies in between the 35 and 12 µg/m^3 identified by the Dutch Health Council.

Based on the directive from the European Parliament and the European Council, annual average concentrations (considering different studies) which would, over a lifetime, equate to an excess risk of contracting leukemia of 1 in a million should be taken as the starting point for developing limit values. Taking this as a precedent and based on the range of unit risks analyzed (6x10^{-6} to 5x10^{-8} per µg/m^3), the corresponding resultant benzene concentration range, which over a lifetime would produce an excess risk of contracting leukemia of 1 in 10^6, would be 0.2 to 20 µg/m^3. The Working Group recommended that this range should be taken as a starting point for developing proposals for a limit value defined as annual average concentration.

From this, the EU panel tabled some of the information related to benzene releases and benzene levels in the environment (Table 4-1 and 4-2), discussed the benefits and costs in a European context for managing the risks of benzene, and summarized three options for consideration.

Figure 4.1: Contribution of main anthropogenic sources of benzene in Europe. This table reveals the fact that automobile emissions are the major benzene contributors rather than industrial emission. Source: EU, 1998.

Sources	Percentage
Vehicular traffic	80 - 85
Petroleum Refineries	0.3 - 1.5
Fuel distribution	2.6 - 6
Chemical industry	1.1 -13
Domesting Heating	3 - 7
Solvent Use	1 – 4

Figure 4.2: Benzene concentration levels observed in European cities. Source: EU, 1998.

Country	Sites	Urban background* ($\mu g/m^3$)
Austria	Several sites	4 - 7
Belgium	Brussel, 68 sites, 1994	1.6 - 11
Italy	Bari (8 sites) 1993	8
	Milan (2 Sites) 1994	
	Rome (1 Site)	
Germany	13 cites (1993)	2 - 5
Sweden	28 cities (1995-96)	2 - 5
The Netherlands	3 cities (1993-94)	2 - 5
Norway	3 cities (1994)	10 - 40
UK	6 cities (1994)	2 – 5

*** Benzene measurements are not always comparable. No data are available on the time periods over which measurements took place.**

The EU panel, as exemplified in the three options presented below, considered that a limit value in the "lowest end of the range" (i.e., 2 to 5 $\mu g/m^3$), would provide a very high level of protection to the population, regardless of location (i.e., hotspot or urban background). Only timing of implementation distinguishes the three options, yet the same range appears to be the preferred target in all cases. Briefly, the EU panel developed these options for consideration by the European Parliament:

> *Option 1* – Considering the fact that people do not spend their entire life time in areas of maximum benzene concentration the working group considered a limit value of 2 to 5 $\mu g/m^3$, to apply in hotspots as well as the urban background, would provide a very high level of protection.
>
> *Option 2* – A more measured approach, with the expectation that the concentrations of benzene in the environment will decline over time, thus a first stage value of 10 $\mu g/m^3$ (2007) can be followed by a second stage limit of 2 to 5$\mu g/m^3$ (2015).
>
> *Option 3* – A long term strategy of gradual reduction of the present level to 2 to 5$\mu g/m^3$ by the year 2015.

On December 13, 2000 the European Parliament set the limit value at 5 $\mu g/m^3$, which must be met by January 2020.

4.1.4 RIVM (2001)

RIVM (National Institute of Public Health and the Environment, The Netherlands) analyzed the dose-response relationship for benzene exposure, taking other jurisdictional comments into account (the WHO, EU). The final regulatory number developed by the Dutch Government is based on their argument below and the recommendation by RIVM.

(1) The unit risk of 6 x 10^{-6} of the WHO does not seem to match the results of meta-analysis by Wong and Raabe (1995), since the AML death cases reported in the study fell short of the number of expected cases.

(2) The carcinogenic effects observed at the occupational level may not be solely driven by genotoxic mechanisms but may also be hematological pre-condition in the workers (e.g., blood and bone marrow toxicity may be involved). The high exposure conditions in the Pliofilm cohort were found to be associated with hematotoxicity. According to IPCS (1993) bone marrow depression or anemia would not be expected to occur in workers exposed for 10 years to TWA benzene concentration of ≤ 3.2 mg/m^3. At these exposure levels, no increased cancer risk has been observed.

(3) Benzene has an unusual genotoxic profile. In spite of its clastogenic potency, gene-mutation hardly occurs. Benzene also does not appear to interact directly with DNA under normal *in vivo* exposure condition.

RIVM derived an ambient air concentration of 0.12 µg/m^3 at an excess lifetime cancer risk of 10^{-6} based on the Rinsky *et al*. (1981) epidemiological data, whereas the Dutch Health Council arrived at an exposure limit of 12 µg/m^3, representative of a 10^{-4} level of increased risk. In a follow-up analysis by RIVM in 1997 (RIVM, 2001), RIVM took the analysis of Wong and Raabe (1995) into account, and noted that continuous life time exposure to 35 µg/m^3 was not associated with an increased risk of cancer. As this value was in agreement with the previously recommended exposure limit of 12 µg/m^3, it was decided to maintain this previously derived value, but update it to represent a 10^{-6} level of increased risk.

4.1.5 World Health Organization (2000)

The WHO noted that the Pliofilm cohort has the most thoroughly studied human data. It was also 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.

The risk estimate for leukemia developed in the WHO Air Quality Guidelines (WHO, 2000) was based on the analysis by Crump (Crump, 1994). Specifically, Crump (Crump, 1994) calculated unit risks for lifetime exposure, represented by a range of unit risks of 4.4 $\times$ 10^{-6} to 7.5 $\times$ 10^{-6} per µg/m^3. The geometric mean of this range of estimates is 6 $\times$ 10^{-6} per µg/m^3, and was adopted as the unit risk for benzene. Thus, the concentrations of airborne benzene associated with an excess lifetime risk of 10^{-4}, 10^{-5} and 10^{-6} are 17 µg/m^3, 1.7 µg/m^3 and 0.17 µg/m^3, respectively.

It should be noted that the WHO Guidelines for drinking water quality of benzene provides a guideline value of 10 µg/L for a 10^{-5} risk estimate for leukemia (WHO, 1993; WHO, 1994 – as referred in WHO, 2003). Using reliable exposure data from an epidemiological study (Rinsky *et al* 1981) and an extrapolation model, a concentration of 0.01 mg/L in drinking water would entail a maximum lifetime risk of one extra case of leukaemia per million people.

4.1.6 UK Environmental Agency (2003)

The inhalation TRV of 0.91µg/kg-day (UK environmental Agency, 2003) was based on an indexed oral dose derived from US EPA (2000) with a leukemic endpoint. The U.K. Expert Panel on Air Quality Standards (EPAQS) accepted that benzene is a genotoxic carcinogen and therefore no absolutely safe exposure level can be defined (EPAQS, 1994). For practical purposes, the Panel noted their belief that a concentration may be proposed at which the risks are "exceedingly small and were unlikely to be detectable by any practicable method" (EPAQS, 1994). In the opinion of EPAQS, it was not possible to make any accurate extrapolation of risks from the relatively high-dose occupational exposure data to the low ambient air exposure levels using formal quantitative risk assessment models. Instead, the UK Environmental Agency recommends a target standard; based on the principle that exposure to genotoxic carcinogens should be kept as low as is reasonably practicable. The epidemiological reports that EPAQS considered most useful in estimating cancer risk were those of Rinsky *et al* (1987) and Wong (1987). EPAQS concluded that the risk of leukemia in workers was not detectable when average exposures over a working lifetime were around 500 ppb (1600 µg /m^3), and the risk would be too small to detect "in any feasible study". In order to take account of the difference between a working lifetime (of about 77,000 h) and a chronological lifetime (of approximately 660,000 h), the figure of 1600 µg /m^3 was divided by 10. A further factor of 10 was used to take account of human variability, to arrive at a recommendation for a starting point air quality standard of 16.0 µg /m^3 (5 ppb) (as a running annual average). EPAQS further recommended a target standard of 1 ppb (3.2 µg /m^3) (as a running annual average), stating the following: "Since benzene is a genotoxic carcinogen and since, in principle, exposure to such substances should be kept as low as practicable".

4.1.7 CalEPA (OEHHA) – Air Toxics Hot Spots Program (2002)

Cancer potency was derived for benzene by the California Department of Health Services (CDHS, 1984) and was re-endorsed by the Air Toxics Hot Spots Program (2002) from the Office of Environmental Health Hazard Assessment.

The CDHS used numerous studies in their analysis – both animal toxicology and human epidemiology. Specifically, CDHS used animal data on Zymbal gland carcinomas of rats exposed via inhalation and gavage (Maltoni *et al*., 1983) and Zymbal gland carcinomas, preputial gland carcinomas and lymphoma or leukemia in male mice or mammary carcinomas in female mice exposed by gavage (NTP, 1984). Epidemiological studies also included inhalation exposure data from Aksoy *et al*., 1974, Aksoy *et al*., 1976, Aksoy, 1977, Infante *et al*., 1977, Ott *et al*., 1978, and Rinsky *et al*.,

1981. A detailed list of the studies used is tabulated in the CalEPA's original document. Summarizing these studies, CalEPA analyzed epidemiological data using a linear non-threshold model to estimate risk, and the animal data were analyzed using a linearized multistage procedure. CDHS (1984) recommended cancer potency values in the range of 24 to 170 x 10^{-6} per ppb to be used in estimating risks from low level exposure to benzene. Assuming that humans breathe 20 m^3/day and weigh 70 kg, the CDHS range of potency values is equivalent to 0.03 to 0.2 $(mg/kg\text{-}day)^{-1}$. In a follow-up, CDHS under Proposition 65 recommended the unit risk of 2.9 x10^{-5} per µg/m^3 to be used to estimate risk specific intake levels from benzene exposure. This corresponds to an annual average concentration of 0.0345 µg/m^3, representing an additional cancer risk of 10^{-6}.

Interestingly, CalEPA established the Public Health Goal (PHG) for benzene, which is a drinking water health-based concentration, based on the Pliofilm cohort (Rinsky *et al*., 1987; Paxton *et al*., 1994; Paustenbach *et al*., 1993). The oral PHG was set at 1.5 x 10^{-4} mg/L, or 0.15 ppb (CalEPA, 2001).

A summary of the aforementioned cancer-based guideline values, the basis of derivation including the date of its endorsement, and any specific comments has been represented in Table 4-3, below.

Figure 4.3: Summary of inhalation unit risk estimates and values corresponding to a 1 in a million risk of cancer death for benzene exposure. Note conversion to similar exponential power units. As well, please note these are values representative of 10^{-6} increased risk (for comparative purpose), and do not necessarily represent the guideline values adopted by the various agencies.

Basis for calculation	Reference	Inhalation Unit Risk	Reference	1 in Million Risk
Pliofilm workers	Rinsky et al., 1987	3.3 x 10^{-6} [a]	Health Canada, 1996	0.3 [b]
Pliofilm workers	Rinsky et al., 1981; 1987	2.2 to 7.8 x 10^{-6}	US EPA, 2000	0.13 - 0.45
Pliofilm workers	Rinsky et al., 1981	8.3 x 10^{-6} [a]	RIVM, 1987	0.12
Pliofilm workers and Epidemiological data (human leukemia)	Rinsky et al.,1981; Wong and Raabe, 1995	5.0 x 10^{-6}	RIVM, 2001	0.2
Pliofilm workers	Crump, 1994	6.0 x 10^{-6}	WHO, 2000	0.17
Epidemiological data (human leukemia)	Numerous studies	29 x 10^{-6}	CalEPA, 2002	0.035
Pliofilm and Epidemiological data (human leukemia)	Numerous studies	0.5 to 6.0 x 10^{-6}	EU, 1998	0.2 - 20

[a] back-calculated from benzene guideline value, for comparison purposes

[b] derived from TD_{05} values, for comparison purposes (TD_{05}/50,000)

Note: Inhalation unit risk estimates represent lifetime risk per µg/m^3 of benzene. Calculated benzene guideline values represent a 10^{-6} lifetime additional cancer risk, in µg/m^3. These calculated values do not necessarily equate to the respective benzene guideline values established by the individual agencies, as they may have landed on acceptable lifetime additional cancer risk levels other than 1 x 10^{-6}.

ACGIH (2009) has updated benzene threshold limit value (TLV) to 0.5 ppm for an industrial exposure scenario. Austin *et al*., (1988) in their discussion, justify the ACGIH approach by comparing four cohort benzene exposure studies including Pliofilm. Austin *et al* (1988) justification and ACGIH approach for benzene does not correspond to a cancer risk of 1 in a million. Additionally, benzene TLV set by ACGIH is a time weighted average under which a person is expected to get exposed at the TLV concentration only during work hours. Industrial workers are expected to use personal protective gears during their work hours. Based on these circumstances the ACGIH number will not be further considered in the development of AAQC.

4.2 Non-carcinogenic Effects

While the development of an Ontario AAQC will focus on the carcinogenic effects of long-term continuous low exposure to benzene, it is worth reviewing the jurisdictional guideline values associated with acute non-carcinogenic effects, as it will become clear that the pathologies underlying these numbers may be related to the carcinogenic effects of benzene. That is, a number of the acute, sub-chronic and chronic non-cancer toxic endpoints involve hematological issues, and thus we cannot dismiss the possibility that such adverse effects may represent early precursor events in leukemia pathology.

4.2.1 US EPA – IRIS (2003)

US EPA (2003) developed a benzene inhalation non-cancer reference concentration of 3.0×10^{-2} mg/m^3, based on the Rothman *et al.* (1996) human exposure studies. Briefly, Rothman *et al*. (1996) conducted a cross-sectional study of 44 workers in Shanghai exposed to a range of benzene concentrations by inhalation in an occupational setting. These exposed workers were age- and sex-matched with unexposed controls. Benzene exposure was monitored with organic vapour passive dosimetry badges worn by the workers. The median 8-hour TWA benzene exposure concentration for the exposed workers was 31 ppm (99 mg/m^3). The exposed workers were divided into 2 groups: those exposed to greater than the median concentration, and those exposed to less than the median concentration. The median concentration in the lower exposed group was 13.6 ppm (43.4 mg/m^3); the median concentration in the higher exposed group was 91.9 ppm (294 mg/m^3). A subgroup of the lower exposed group consisted of 11 individuals who were not exposed to >31 ppm (median of all exposed workers) at any point in the study; the median for this subgroup was 7.6 ppm (24 mg/m^3).

All 6 blood parameters evaluated were significantly different in the high exposed group: white blood cell count (WBC), red blood cell count (RBC), absolute lymphocyte count (ALC), hematocrit, and platelets, were all significantly decreased; mean corpuscular volume (MCV) was significantly increased. ALC was used as the critical endpoint since it is thought to be a “sentinel” for a cascade of hematological and biological changes that might be expected to result in more profound examples of benzene poisoning observed in other cohort studies (Dosemeci *et al*., 1996). Benchmark concentration (BMC) modeling was conducted on the ALC data using a continuous linear model after the exposure levels were logarithmically transformed. The BMC was calculated to be

13.7 ppm (8-hour TWA); the lower 95% confidence limit (BMCL) was calculated as 7.2 ppm (8-hour TWA) and was chosen as the point of departure for the RfC derivation (US EPA, 2000). The BMCL 8-hour TWA was adjusted by converting ppm to mg/m^3 and by adjusting the 8-hour TWA to an equivalent continuous environmental exposure using default respiration rates where 10 m^3 is the human occupational volume of air inhaled in an 8-hour work shift and 20 m^3 is the human ambient volume of air inhaled in a 24-hour day (US EPA, 1994). The $BMCL_{ADJ}$ was 8.2 mg/m^3. The RfC was derived by dividing the $BMCL_{ADJ}$ by the composite UF of 300 (3 for less severity in effect-level [USEPA considers UF 3 for less serious effects and UF 10 for more series effects], 10 for intraspecies differences, 3 for subchronic-to-chronic extrapolation, and 3 for database deficiencies). This resulted in an RfC of 3×10^{-2} mg/m^3 (30 µg/m^3).

US EPA also conducted a comparative analysis using the subchronic experimental haematological data from animal inhalation study by Ward *et al*. (1985). A LOAEL of 300 ppm and a NOAEL of 30 ppm were observed. It is worth noting that other studies in experimental animals have observed significant hematological effects at benzene exposure of 10-25 ppm, which is lower than the NOAEL of 30 ppm from the Ward *et al*. (1985) study. However, these studies did not have sufficient data for dose-response modeling. Baarson *et al*. (1984), for example, exposed male C57BL/6J mice (five/group) to 10 ppm benzene, 6 hours/day, 5 days/week, for 178 days and observed statistically significant reductions in blood lymphocytes at each of the three monitoring time points (32, 66, and 178 days) when compared to controls. The magnitude of the reduction in lymphocytes ranged from about 53% at 32 days to about 68% at 178 days. Cronkite *et al*. (1985) exposed male and female C57BL/6 BNL mice to various concentrations of benzene 6 hours/day, 5 days/week for 2 weeks and observed no decrease in blood lymphocytes at 10 ppm, but they did observe a statistically significant reduction of about 21% at 25 ppm as compared to controls (5-10 mice/group). Thus, lower RfCs than those calculated above from the Ward *et al*. (1985) study are possible, based on other experimental animal results. In the most extreme case, using a LOAEL of 10 ppm and an overall UF of 3000 yields a $LOAEL_{ADJ}$ of 5.7 mg/m^3 and an RfC of 2×10^{-3} mg/m^3 were arrived.

4.2.2 ATSDR (2007)

ATSDR (2007) described non-carcinogenic Minimum Risk Level (MRL) values for different duration of exposures (acute, intermediate and chronic), for inhalation. Oral MRL values have not been developed by ATSDR for benzene, as it was deemed there was a paucity of experimental evidence for quantitative risk assessment.

Acute inhalation exposure

The acute MRL is based on an inhalation study (Rozen *et al*., 1984) where male C57BL/6J mice (7-8 per group) were exposed to benzene (0, 10.2, 31, 100, or 300 ppm – equivalent to 0, 33, 100, 324, 975 mg/m^3) in whole-body dynamic inhalation chambers for 6 hours/day for 6 consecutive days. Erythrocyte counts were depressed in C57BL/6 mice only at 100 ppm (324 mg/m^3) and 300 ppm (975 mg/m^3). The 10.2 ppm (33 mg/m^3) exposure level resulted in significant depression of femoral lipopolysaccharide-

induced B-colony-forming ability in the absence of a significant depression of total numbers of B cells. At 31 ppm (100 mg/m^3), splenic phytohemaglutinin-induced blastogenesis was significantly depressed without a concomitant significant depression in numbers of T-lymphocytes. Peripheral lymphocyte counts were depressed at all exposure levels. These results demonstrate that short-term inhaled benzene even at low exposure concentrations can alter certain immune associated processes.

The LOAEL of 10.2 ppm (33 mg/m^3) was adjusted for intermittent exposure by multiplication with 6/24 to correct for less than a full day of exposure, resulting in a $LOAEL_{ADJ}$ of 2.55 ppm (8.1 mg/m^3). According to US EPA (US EPA, 1994), the $LOAEL_{ADJ}$ should be converted into a human equivalent concentration (HEC) using the ratio of the blood: gas partition coefficient of the laboratory animal to the human value. In this case, the animal blood: gas coefficient is greater than the human blood: gas coefficient, so that a default value of 1 is used. Therefore, the $LOAEL_{HEC}$ is equal to the $LOAEL_{ADJ}$, which is 2.55 ppm (8.1 mg/m^3). The $LOAEL_{HEC}$ of 2.55 ppm (8.1 mg/m^3) is divided by the composite UF of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, 10 for human variability), yielding an acute MRL of 0.009 ppm (28.75 µg/m^3).

Intermediate inhalation exposure

The intermediate MRL is based on the experiment conducted by Rosenthal and Snyder (1987) where male C57Bl/6 mice were exposed to 10, 30, or 100 ppm of benzene (equivalent to 32.4, 97, and 325 mg/m^3) by inhalation 6 hours/day, 5 days/week for 20 exposure days. The number of lymphocytes and their functional capacities were evaluated in spleens of exposed mice. Following the 20 days of exposure, functional capacity of splenic lymphocytes was evaluated in two *in vitro* assays: mixed-lymphocyte culture (MLC) and ^{51}Cr-release cytotoxicity assay. Measured mean daily benzene concentrations in the 10, 30, and 100 ppm groups were 11.1 (±1.5) ppm, 29.5 (±4.4) ppm, and 99.7 (±7.0) ppm, respectively. No changes were observed in the relative proportions of splenic leukocytes, in the percentage of T-cell subsets or in the ratio of T-helper and T-suppressor cells, even at the highest exposure level. Therefore, the functional assays could be normalized for particular lymphocyte populations by using equal numbers of splenic cells. MLC is an *in vitro* measure of alloreactivity (capacity to mount an immune response against foreign antigens). The MLC activity of spleen lymphocytes from 10- and 100-ppm mice was delayed on days 2–4 of culture (relative to air-exposed controls), indicating that benzene exposure causes impaired *in vitro* alloreactivity (data for the 30-ppm mice were not included in the reported results). This delayed alloreactivity was not due to spleen suppressor cells. The lymphocyte cytotoxic function evaluated in the Cr-release assay was also altered; splenic lymphocytes from 100-ppm mice had a significantly reduced lysing capacity. The results indicate that inhalation exposure of mice to benzene has an immunosuppressive effect on *in vitro* alloreactivity and cytotoxicity of splenic lymphocytes.

The LOAEL of 10 ppm (32 mg/m^3) was adjusted for intermittent exposure by multiplication with 6/24 to correct for less than a full day of exposure and with 5 days/7 days to correct for less than a full week of exposure, resulting in a $LOAEL_{ADJ}$ of 1.8

ppm. According to US EPA (1994), the $LOAEL_{ADJ}$ must be converted into a human equivalent concentration (HEC) using the ratio of the blood: gas partition coefficient of the laboratory animal to the human value. In this case, the animal blood: gas coefficient is greater than the human blood: gas coefficient, so that a default value of 1 is used. Therefore, the $LOAEL_{HEC}$ is equal to the $LOAEL_{ADJ}$, which is 1.8 ppm. The $LOAEL_{HEC}$ of 1.8 ppm (5.75 mg/m^3) is divided by the composite UF of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, 10 for human variability), yielding an intermediate duration MRL of 0.006 ppm (19.17 $\mu g/m^3$).

Chronic inhalation exposure

The chronic MRL is based on a cross-sectional study of 250 workers (approximately two-thirds female) exposed to benzene at two shoe manufacturing facilities in Tianjin, China, and 140 age- and gender-matched workers in clothing manufacturing facilities that did not use benzene (Lan *et al*., 2004) (Table B - Appendix) . The benzene exposed workers had been employed for an average of 6.1 ± 2.9 years. Benzene exposure was monitored by individual organic vapour monitors, 5-or-more times during sixteen months prior to phlebotomy. Post-shift urine samples were collected from every worker. Urinary benzene concentrations were highly correlated with mean individual air levels. Benzene was not found (detection limit 0.04 ppm) in workplace and home air samples of control workers taken at three different time periods. Study subjects were categorized into four groups (140 controls, 109 at <1 ppm, 110 at 1 to <10 ppm, and 31 at ≥10 ppm) according to mean benzene exposure levels measured twice during the month prior to phlebotomy. Of the 250 exposed workers, 109 were exposed to <1 ppm benzene. Each of these individuals worked at the larger of the two facilities included in the study. Exposure concentrations were generally higher at the smaller facility due to a less adequate ventilation system. Complete blood count (CBC) and differential were analyzed mechanically. Coefficients of variation for all cell counts were <10%.

The mean 1-month benzene exposure levels in the controls, <1 ppm (3.2 mg/m^3), 1-10 ppm (32 mg/m^3), and ≥10 ppm were <0.04, 0.57 ± 0.24, 2.85 ± 2.11, and 28.73 ± 20.74 ppm, respectively. White blood cells (WBCs) and platelets were significantly decreased by 8-15% in the lowest exposure group (<1 ppm) compared to controls. The mid- and high-exposure groups also had decreases in WBCs and platelets; decreases in the high-exposure group ranged from 15-36%. Lymphocyte subset analysis revealed significantly decreased CD4+-T cells, CD4+/CD8+ ratio, and B cells. Hemoglobin concentrations were significantly decreased only within the highest (≥10 ppm) exposure group.

In order to evaluate the effect of past benzene exposures on the hematological effects observed in this study, the authors compared findings for a group of workers who had been exposed to <1 ppm benzene over the previous year (n = 60) and a subset who also had <40 ppm-years lifetime cumulative benzene exposure (n = 50). The same cell types were significantly reduced in these groups, but the percent change was not reported. These data suggest that one month exposure to benzene results in hematotoxicity. To demonstrate that the observed effects were attributable to benzene, significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells were

noted in a subgroup (n = 30; mean 1-month exposure level of 0.29 ± 0.15 ppm) of the <1 ppm group for which exposure to other solvents was negligible.

Benzene-induced decreased B cell counts was selected as the critical effect for BMD modeling because it represented the highest magnitude of effect. The Hill BMD model was the only model to provide an adequate fit of the data set when comparing plots of observed versus expected values for B cell counts. A benchmark response (BMR) of 0.25 standard deviations were selected because it resulted in a $BMC_{0.25SD}$ of 0.42 ppm with a 95% lower confidence limit ($BMCL_{0.25SD}$) of 0.10 ppm. This value is below the mean exposure level of the lowest exposure group (0.57 ppm). The $BMCL_{0.25SD}$ of 0.10 ppm was selected as the point of departure for deriving a chronic inhalation MRL. The $BMCL_{0.25SD}$ of 0.10 ppm was adjusted from the 8-hour TWA to continuous exposure (multiplication by 8/24) and for 6 days to 7 days a week. This results in an adjusted $BMCL_{0.25SD\text{-}ADJ}$ of 0.03 ppm (95.8 µg/m^3). This value was divided by an UF of 10 to account for human variability, yielding the chronic inhalation MRL of 0.003 ppm (9.58 µg/m^3).

4.2.3 CalEPA (OEHHA) (2000)

The State of California developed an acute Reference Exposure Level (REL) based on the developmental effects of benzene in rats (Coate *et al*., 1984). Here, pregnant rats were exposed by inhalation to 0, 1, 10, 40 or 100 ppm (0, 3.24, 32.4, 129.6 or 324 mg/m^3) benzene for 6 hours/day, on gestational days 6 to 15. Mean fetal body weights were significantly decreased at the 100 ppm exposure; no teratogenic, fetotoxic, or maternally toxic effects were observed at 40 ppm or less. The NOAEL was 40 ppm (128 mg/m^3), and was extrapolated to a 1-hour exposure (using the equation $C^n * T = k$, where n = 2) value of 100 ppm. A composite UF of 100 (10 for interspecies variability, 10 for intraspecies variability) was applied, resulting in a REL of 1.0 ppm (3.24 mg/m^3). However, the acute REL provided by CA OEHHA is for a 6-hour exposure, so that the UF of 100 was directly applied to the NOAEL of 40 ppm, resulting in a 6-hour acute REL of 0.4 ppm (1300 µg/m^3).

A chronic REL was developed based on hematologic parameters in a cohort of 303 male workers exposed to benzene for 1-21 years in a refinery from 1952-1978 (Tsai *et al*., 1983). A total of 1400 hematologic tests and 900 blood chemistry tests were taken between 1959 and 1979. Benzene exposures were determined by personal monitors. Data from 1394 personal samples indicated that 84% of samples were <1 ppm. The median air concentration of benzene was 0.53 ppm in the work areas of greatest exposure. The average length of employment was 7.4 years; 32% of the workers had worked for more than 10 years. Mortality from all causes and from circulatory system diseases was significantly below expected values, indicating a healthy worker effect. Total and differential WBC counts, hemoglobin, hematocrit, RBCs, platelets, and clotting times were within the normal range for this cohort.

The NOAEL identified in this study is 0.53 ppm (1.69 mg/m^3), with exposures of 8 hours/day, 5 days week (Tsai *et al*., 1983). This exposure was converted to equivalent continuous environmental exposure using default respiration rates (US EPA, 1994). This adjustment results in a human equivalent concentration of 0.19 ppm (607 mg/m^3).

Applying an UF of 10 to account for intraspecies variability results in a chronic REL of 0.02 ppm (20 ppb; 60 µg/m^3).

For comparison purposes, Cody *et al*. (1993) reported significant hematological effects, including decreased RBC and WBC counts, in 161 rubber workers exposed to median peak concentrations of 30-54 ppm during the year 1948. The 30 ppm (96 mg/m^3) was considered a LOAEL. Exposure time adjusting this exposure results in a LOAEL of 10.7 ppm (34 mg/m^3). Applying a composite UF of 1000 (10 for the use of a subchronic study, 10 for the use of a LOAEL instead of a NOAEL, and for human variability) results in a REL of 0.01 ppm (10 ppb; 30 µg/m^3). For further comparison, a REL was developed based on a chronic inhalation study in mice by Baarson *et al*. (1984). This study found that mice exposed for 6 hours/day, 5 days/week to 10 ppm for 6 months, exhibited bone marrow progenitor cells that were markedly suppressed. Conversion of this exposure to an equivalent continuous exposure results in a concentration of 1.8 ppm. Applying a composite UF of 300 (3 for interspecies variability, 10 for intraspecies variability, and 10 for the estimation of a NOAEL from a LOAEL) results in a REL of 6 ppb (20 µg/m^3).

4.3 Agency-Specific Air Quality Guidelines

In revising the air quality standards for Ontario, the Ministry of the Environment is considering risk assessments, standards and guidelines used by environmental agencies world-wide. This document lists the air quality guidelines and standards developed, recommended, or adopted by the States of California, Louisiana, Massachusetts, Michigan, New Jersey, New York, North Carolina, and Texas, and the countries of France, UK, The Netherlands, Sweden, and the U.S. (via IRIS and ATSDR programs). Brief summaries of these benzene regulatory values are presented in Table 4.4.

Following Page:

Table 4.4: Comparison of benzene air quality guidelines and standards for various world-wide agencies. Note that the values are separated into cancer and non-cancer endpoints

Jurisdiction	Agency	Benzene Guideline Value ($\mu g/m^3$)	Date	Comments
CANCER ENDPOINTS				
Based on USEPA inhalation cancer risk				
Louisiana	DEQ	12 (8 hour AAS)	2001	Based on a 1 x 10^{-4} additional cancer risk
Massachusetts	DEP	0.12 (annual average)	1995	Based on a 1 x 10^{-6} additional cancer risk
Michigan	DEQ	0.1 (annual average)	1987	Based on a 1 x 10^{-6} additional cancer risk
New Jersey	DEP	0.13 (annual average)	2000	Based on a 1 x 10^{-6} additional cancer risk
New York	DEC	0.13 (annual average)	2000	Based on a 1 x 10^{-6} additional cancer risk Ambient guidelines used for permitting
North Carolina	DENR	0.12 (annual average)	2000	Based on a 1 x 10^{-6} additional cancer risk
Texas	TCEQ	4.5 (annual average)	2007	Long-term effect screening level used for permitting at 10^{-5} additional cancer risk
Based on EU cancer risk value				
United Kingdom	N/A	3.2 (recommended) (annual average)	2000	Based on leukemia risk
Derived by jurisdiction				
The Netherlands	N/A	12 (annual limit value)	2001	Based on 10^{-6} additional cancer risk

Jurisdiction	Agency	Benzene Guideline Value ($\mu g/m^3$)	Date	Comments
France	N/A	10 (immediate); 2 (target)	1997	Based on leukemia at 10^{-5} additional cancer risk
Sweden	N/A	1.3 (long-term average)	1993	Based on 10^{-5} additional cancer risk
California	OEHHA	0.0345 (annual)	2000	Based on a 1 x 10^{-6} additional cancer risk
NON-CANCER ENDPOINTS				
Derived by jurisdiction				
California	N/A	60 (chronic REL)	2000	Chronic Reference Exposure Level (non-cancer)
Massachusetts	DEP	1.74 (24 hr average)	2001	Based on NIOSH TWA of 3.19 mg/m^3 Threshold effect level
New Jersey	DEP	19 (24 hr RfC)	1994	Based on maternal fetal or developmental effects IRIS unit risk
New Jersey	DEP	60 (annual RfC)	2001	Based on USEPA chronic REL
IRIS	US-EPA	30 (RfC)	2003	Based on Rothman *et al*,. 1996 epidemiological study
ATSDR (acute MRL)	N/A	28.75	2005	Based on depressed erythocyte counts in mice (Rozen et al., 1984)
ATSDR (intermediate MRL)	N/A	19.17	2005	Based on immunodepressive effects in mice (Rosenthal and Snyder, 1987)

Jurisdiction	Agency	Benzene Guideline Value ($\mu g/m^3$)	Date	Comments
ATSDR (chronic MRL)	N/A	9.58	2005	Based on hematological effects in workers (Lan et al., 2004)

4.4 General Comments

Upon reflecting on the various jurisdictional summaries, it is clear that whether one examines a carcinogenic or non-carcinogenic endpoint, a unifying theme is the adverse effects on hematopoietic system as a result of benzene inhalation exposure. Specifically, this refers to either the critical endpoints of leukemia (cancer endpoint) or alteration in blood cell count or activity (non-cancer endpoints). While acute myeloid leukemia (AML) has been strongly suggested as the manifested leukemia, NTP (NTP, 2005) refers to the work by Savitz and Andrews (1997) when suggesting that the evidence supports an association between benzene exposure and leukemia in general, rather than with a specific leukemia subtype, such as AML. Interestingly, while Wong and Raabe (1995) also reported an increase in acute lymphocytic leukaemia (ALL) in their meta-analysis, death cases of AML were observed to be less than the expected outcome. Regardless, while leukemia – either its various subtypes or as a group – may be associated with benzene exposure, only AML data are useful for a quantitative analysis (i.e., only AML data, of all the leukemia data, is well-controlled and extensive enough to allow quantitative analysis).

CalEPA originally developed the cancer guidelines that correspond to 0.0345 $\mu g/m^3$ of benzene in air, representing an additional cancer risk of 10^{-6}. In 2002, the Air Toxics Hot Spots Program from the Office of Environmental Health Hazard Assessment re-endorsed the same assessment. This assessment includes Pliofilm studies and analysis only prior to Paxton *et al*., (1994) or Paustenbach *et al*., (1993) or the Rinsky (1987) analysis. However this analysis also includes some of the prior animal and human data. Interestingly, the drinking water PHG for benzene was originally derived in 2001 and used route-to-route extrapolation of the Pliofilm inhalation study including, the analysis by Paxton *et al*., (1994) or Paustenbach *et al*., (1993) or the Rinsky (1987). Because of the inconsistencies, in the approaches for guidelines developed for different routes of exposure, CalEPA analysis was not further considered in MOE analysis.

RIVM originally derived an ambient air concentration of 0.12 $\mu g/m^3$ at an excess lifetime cancer risk of 10^{-6} based on the Rinsky *et al*. (1981) epidemiological data. Dutch Health Council arrived at an exposure limit of 12 $\mu g/m^3$, representative of a 10^{-4} level of increased risk. However, in a follow-up analysis, RIVM in 1997 (RIVM, 2001) took the analysis of Wong and Raabe (1995) into account, and noted that continuous life time exposure to 35 $\mu g/m^3$ was not associated with an increased risk of cancer. As this value was in agreement with the previously recommended exposure limit of 12 $\mu g/m^3$, it was decided to maintain this previously derived value, but was now considered to

represent a 10^{-6} level of increased risk. RIVM based its later decision on the Wong and Raabe paper, which will be analyzed in this MOE document. Hence, the RIVM approach was further considered.

The proposed standards both by US EPA (2000) and EU (1998) are widely used by different jurisdiction in developing their own regulatory numbers. These proposed approaches need to be analyzed further using the current knowledge in benzene toxicology – that is, *an appreciation of benzene toxicity at low environmental doses*.

5.0 Considerations for the Development of Ambient Air Quality Criteria for Benzene

In developing air standards for Benzene, the Ministry is weighing on the scientific evidence from the toxicological evaluation of the significant endpoints from benzene exposures, the quality of the risk assessments from other jurisdictions for the derivation of their respective air guidelines, and the science-related comments received from stakeholders.

5.1 Responses of Stakeholders to the Science Discussion Document

In January 2009, under the Standards Plan for air standards development (MOEE, 1996; MOE, 1999), the Ministry distributed the Science Discussion Document for benzene (MOE, 2009). The Ministry requested scientific input regarding the toxicological information examined by the Ministry and, comments on the strengths and weaknesses of the possible paths towards the development of the air standard for benzene. On February 11, 2009, the Ministry hosted a Pre-consultation Science Meeting at Queen's Park, Toronto for face-to-face science discussion with interested stakeholders. Following the meeting, the MOE received written submissions from various stakeholders, representing the petroleum production industry, the steel manufacturing industry, and a number of public health units.

A summary of the key comments from the stakeholders is outlined below, and were taken into considered in arriving at the proposed air standard. Some of the key comments are discussed and incorporated into following sections, or earlier sections of this rationale document.

- Inconsistency in hematological results at environmental benzene exposure concentrations suggests only inconclusive evidence for benzene induced hematotoxicity to the general public

- The possibility of a functional threshold for hematotoxic effects

- Concerns of the difficulty in the utilization of urinary biomarkers as evidence of exposure (i.e., metabolites) at very low environmental concentrations, in understanding the dose-response nature of benzene

- Michaelis-Menton kinetics follows a non-linear pattern. Benzene metabolism typically follows the Michaelis-Menton kinetics. Linear extrapolation of risk from high to low exposure concentration does not support the non-linear Michaelis-Menton pattern

- The occurrence of certain genotoxic effects (e.g., micronucleus formation, global DNA hypomethylation, DNA adduct formation) at doses higher than ambient air concentrations has been recognized. However, a direct causal link between these events and AML has not been established

- The SDD suggests that the presence of chemicals including toluene would decrease the metabolism of benzene and hence, the metabolite concentrations would also be expected to be low (i.e., chemical mixture effects). However, benzene-induced micronucleus formation is increased by the presence of toluene at high concentration in experimental mice. This finding contradicts the chemical mixture effects discussed in the SDD

- Lack of causal specificity of global DNA hypomethylation as one of the cellular effects of benzene exposure, as well as a lack of genetic damage in target tissue

- Lack of reverse mutation in *Salmonella typhimurium* for exposure to benzene or its metabolites and its implications to human exposure

- The potential for elevated risk due to benzene exposure in human sub-populations (e.g., children)

- Variations in benzene metabolism due to genetic differences may predispose certain individuals to increased risks from benzene exposure

- The applicability of using occupational exposure limits rationale for the development of an AAQC for benzene

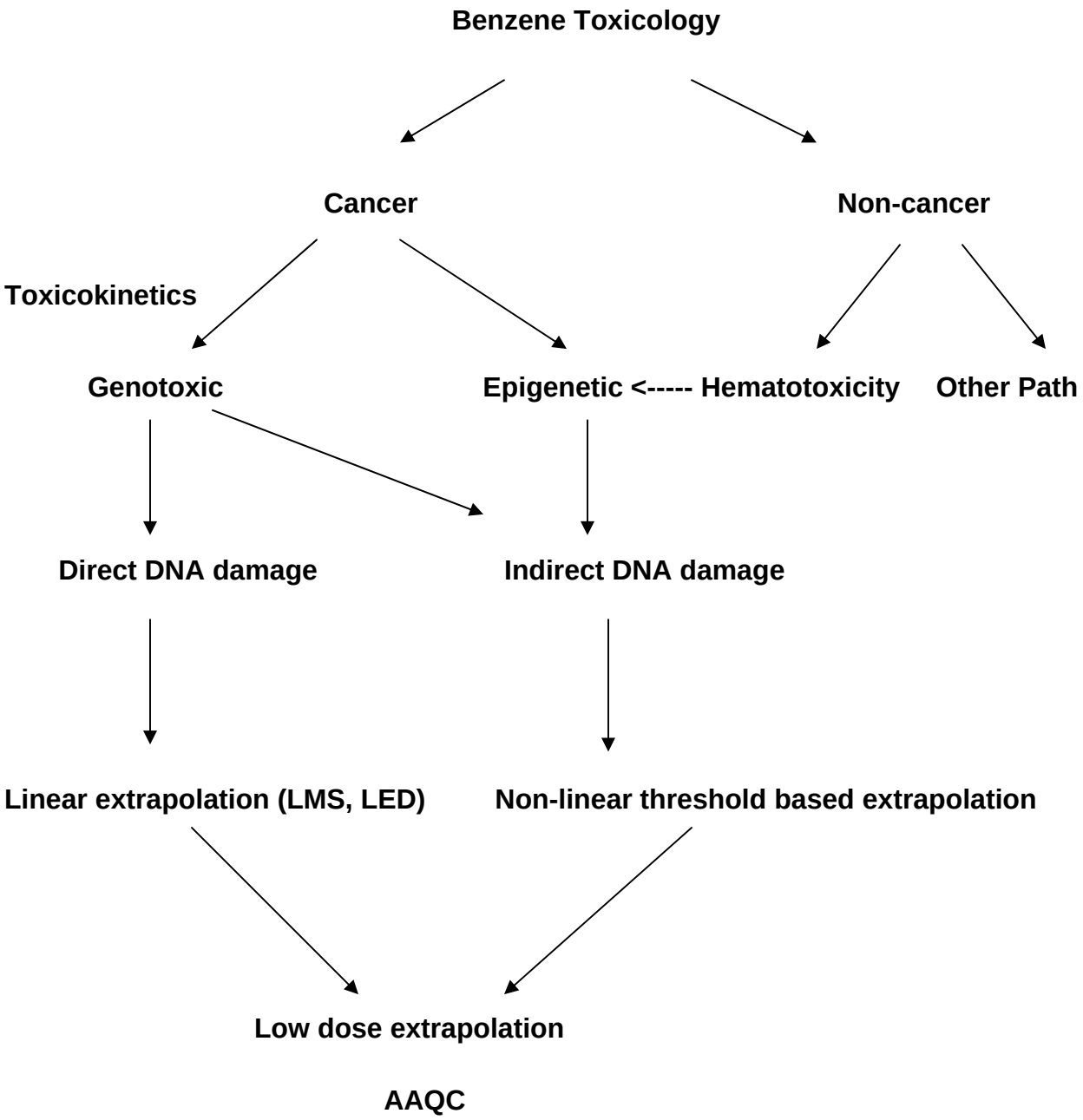
5.2 Strategies in the development of benzene AAQC

Dose-response analysis and extrapolation of risk to low environmental concentrations are the key components in the development of an AAQC for benzene. For the comprehensive understanding of these key components, a framework has been developed (Figure 5.1). This framework analyzes and summarizes the current science of benzene toxicology, and takes the key stakeholder comments in consideration. Specifically, the framework elaborates on endpoint analyses (e.g., cancer and non-cancer), toxicokinetic interpretations (e.g., metabolites), and toxicodynamic impacts (e.g., genotoxic, epigenetic, etc.), dose-response analyses, low dose extrapolation of risk, and culminates with final considerations.

In benzene cohort studies, mortality is an observed frank toxicological effect (death), usually associated with high exposure concentrations. Mortality as a result of exposure to benzene might not be easily observed at ambient air concentrations but that does not represent a threshold for benzene toxicity. In fact, the risk of benzene at low doses may involve a series of clinical and preclinical events that can be characterized with an understanding of toxicokinetics and the toxicodynamics of these events.

Cancer pathology involves a chain of events that can be observed either as clinical or preclinical steps. In a mutagenic pathway, the DNA base pair pattern is affected (initiation) which leads to altered genetic signalling during cell proliferation. If this clonal expression continues (progression) a mutated cell line (e.g. carcinogenic) will develop leading to gross tissue pathology (e.g. cancer type – AML). Understanding of the toxicokinetic and toxicodynamic mechanisms should help in revealing the benzene cancer toxicology.

Figure 5.1: Framework for the development of an AAQC for benzene



5.3 Selection of Critical Endpoint

Both non-carcinogenic and carcinogenic endpoints have been analyzed in depth by various regulatory agencies, and are summarized in Chapter 4. Selection of the most critical effect will be determined by:

(i) The qualitative assessments of the systemic toxicological effects of benzene

(ii) The quantitative assessment of the exposure-to-effect relationship for different endpoints (i.e., cancer and non-cancer) from other jurisdictions

5.3.1 Non-carcinogenic Endpoints

While effects on central nervous system (CNS) were one of the critical endpoints identified in acute toxicity studies, chronic benzene toxicity on the hematopoietic system was considered the critical non-carcinogenic endpoint by most agencies. Hematopoietic toxicity occurs at lower exposure concentrations than those that elicit the CNS effects, and most of the regulatory non-cancer health-based concentrations are derived from the effect on the hematopoietic system. Moreover, the hematopoietic responses are based on objective quantifiable measurements (i.e., blood cell quantification) rather than on potentially subjective neurological tests. Furthermore, while CNS effects are reversible upon cessation of exposure, most of the hematopoietic effects are irreversible. As well, it has been hypothesized that non-carcinogenic hematopoietic effects might actually be on a continuum towards carcinogenesis. For example, evidence from extensive chromosomal studies has suggested that exposure to benzene-induced chromosomal aberrations in bone marrow cells may be a predictor of future leukemia risks (Forni, 1996; Au *et al*., 2002).

5.3.2 Carcinogenic Endpoints

Benzene is a well established systemic carcinogen, showing effects on multiple target organs, with varying degrees of severity. Leukemia has been observed as the severe effect in many epidemiological and animal studies following benzene exposure. Specifically, while several types of leukemia have been observed in populations exposed to benzene, the majority of epidemiological studies suggest that AML is the subtype of leukemia that is the specific critical endpoint. The pathology of these cancers is thought to be based on the actions of the metabolites of benzene – hence, the analysis of the mechanism of benzene metabolite formation is important. Based on toxicokinetic and toxicodynamic analyses, a dose-response (Chapter 3) relationship is observed only in leukemia. Specifically, quantitative dose-response analysis for benzene exposure is only possible for AML, which has been used by other agencies in developing air quality criteria.

5.3.3 Endpoint Selection

When both carcinogenic and non-carcinogenic endpoints are considered, the carcinogenic effects are thought to occur at comparably lower exposures (Table 4.4). This finding has been deduced from the examination of human epidemiological and animal studies, which consistently point towards the importance of carcinogenesis as the most toxic process due to benzene exposure. Thus, regulatory agencies have developed more conservative carcinogenic guidelines using AML as an endpoint, as compared to their non-carcinogen guideline values. As indicted in table 4.4, most of the jurisdictions have cancer-based guidelines below 1 μg/m^3, at a one in one million equivalent risk level, except those from the EU. In contrast, the non-cancer guidelines are well above this level [e.g. 9.53 μg/m^3 of ATSDR (2007)].

Comprehensive analysis of non-cancer endpoints including neurological and hematological effects reveals the fact that hematological effects have a threshold below the neurological effects. However, there remains the possibility that such hematological effects may represent a continuum of events, ultimately manifesting in more severe effects such as leukemia, acknowledging that such a link to AML has not been established. Leukemia is frequently observed in animals and humans exposed to benzene. On this basis, most of the jurisdictions derive their air guidelines based on the carcinogenic effects of benzene.

With the above considerations, and in agreement with all stakeholders, the MOE has determined leukemia (e.g., AML) as the critical endpoint in the development of an AAQC for benzene.

5.4 Toxicokinetics Consideration

Both cancer (see Sections 3.5, 3.6) and hematological effects (e.g., pancytopenia – see Section 3.3) are primarily associated with the metabolites of benzene. The dose-response relationship of these adverse effects may be characterized by the formation of the metabolites (toxicokinetics). These events have been demonstrated to occur at benzene concentrations encompassing occupational exposures, to urban ambient air concentrations (i.e., ppm to ppb range). The data derived from such studies may provide an understanding of the toxicokinetics, toxicodynamics, and/or dose-response characteristics over a range of exposures, including those found in ambient air.

Cytochrome P450 (CYP2E1) is the major rate limiting enzyme in the toxicokinetics of benzene (see Section 3.1.3). CYP2E1 is a high-affinity, low-capacity isozyme. Enzyme-substrate complex formation and subsequent production of metabolites involving CYP2E1 follows the traditional first order Michaelis-Menton kinetics. Indeed, the Michaelis-Menton equation fits into non-linear dose-response relationship (Travis *et al.* 1990a, Travis *et al.* 1990b). However, this non-linear toxicokinetic pattern is observed at high benzene exposure concentrations (e.g., occupational exposure such as Pliofilm) above the point of enzyme-substrate saturation. In fact, at exposure concentrations below saturation (e.g., ambient air), linear enzyme kinetics is observed (see Section 3.8), noting the process of enzyme saturation may start at concentrations approaching 3200 μg/m^3 (Rappaport and Kupper, 2004; Rappaport *et al*., 2005).

Interestingly, the higher doses of Pliofilm occupational exposure should be in the metabolic saturation concentration range, meaning that an extrapolation of risk from occupational exposures (benzene metabolites are carcinogenic) could under-estimate risk at lower ambient air doses (i.e., an example of possible supra-linearity). Thus, it has been suggested that the biologically effective dose of toxic benzene metabolites should be proportionally greater in persons exposed to lower (e.g., environmental exposure) rather than higher (e.g., occupationally exposure) levels of benzene (Rappaport *et al*., 2002a; Rappaport *et al*., 2002b; Rappaport *et al*., 2009).

Rappaport *et al*. (2009) have recognized an additional high affinity pathway well below 3200 $\mu g/m^3$, to as low as 3.2 $\mu g/m^3$ of benzene exposure, supporting earlier work [Rappaport *et al*., (2002a); Rappaport *et al*., (2002b); Rappaport *et al*., (2005); Kim *et al*., (2006)]. Rappaport *et al*. (2009) examined low dose metabolism in humans (Section 3.8), and revealed a better enzyme kinetic curve fitting of the data if a bi-variant Michaelis-Menton equation is considered (e.g. differences in V_{max} and K_m). This biphasic metabolic model adds support to the fact that there may be two points of saturation at different exposure concentrations of benzene. This basically refers to the fact that the metabolite production at well below 3200 $\mu g/m^3$ may be faster due to non-saturation of the additional high affinity pathway. In such instances, metabolite production with unsaturated enzyme complex at low benzene exposure level (< 1 ppm or 3200 $\mu g/m^3$) would increase the metabolites concentration up to an estimated level of three times (see Section 3.8 and Rappaport *et al.* 2009), when compared to linear extrapolation. As such, this may imply a supralinear toxicokinetic pattern.

Urinary metabolites of benzene have been used as specific biomarkers (e.g. S-phenylmercapturic acid) of benzene exposure. However, at low exposure doses the metabolite excretion is negligible. Coupled with metabolite volatilization, it becomes difficult to analytically quantify benzene exposure using urinary metabolites as a biomarker of exposure at low doses. However, high metabolite concentrations can be measured in blood samples from low benzene exposures (Kim *et al*., 2006; Chanvaivit *et al*., 2007; Rappaport et al., 2002a) and provide a more reliable sample medium than urine in studying the toxicokinetics of benzene metabolism. The validity of the findings of Rappaport *et al*. (2009) relies mainly on the use of metabolites in blood samples as the biomarker of exposure rather than using urine samples.

Below the limit of enzyme saturation, CYP2E1 has the capacity to metabolize benzene, in the presence of toluene, ethyl benzene, xylenes – a condition likely to be observed in environmental exposure concentrations (see Sessions 3.1.3 and 3.1.5). MOE validated and supported this phenomenon with a PBTK model using the human benzene blood concentration data of Chanvaivit *et al*. (2007) (Figure 3.7).

However, nearing or above saturation, competition of these chemicals for CYP2E1 will likely occur and therefore may reduce the formation of the benzene metabolites and the subsequent toxicodynamics consequences. Such a phenomenon is likely to occur at occupational levels (e.g., petroleum processing facilities). This observation has been validated by a PBTK chemical mixture model (see Section 3.1.5 and Table 3-1) where chemical interactions occurred at high concentrations – a condition mimicking occupational exposure. For this reason, the interpretation of benzene related mortality

data from epidemiological studies of the petroleum industries may be confounded by the presence of other chemicals (e.g., toluene). These confounding factors limit the utility of petrochemical studies for deriving ambient air quality criteria.

Wetmore *et al*., (2008) observed that toxicodynamic effects of benzene in terms of micronucleus formation are not influenced by the presence of toluene at very high concentrations (160 mg/m^3 and 320 mg/m^3). Interestingly, this effect was observed in the micronucleated polychromatic erythrocytes (a form of red blood cell) but not reported in the lymphocytes which are the target cell for hematotoxicity and/or leukemia.

5.5 Toxicodynamic Consideration

The toxicological effects of benzene on humans (i.e., toxicodynamics) at various exposure concentrations have been summarized in Chapter 3. Various metabolites of benzene are responsible for the toxicodynamic effects of benzene. Total metabolite concentration per unit exposure of benzene is greater at low benzene exposures, as compared to high exposures (see Section 3.8; 5.3). Thus, the metabolite profile observed at higher occupational exposure doses (i.e., the amount of different types of metabolites) is not likely the same profile observed at low doses. In this regard, if low dose exposure to benzene favours an increase in the production of the toxic metabolites hydroquinone and muconic acid relative to benzene, this may have significant toxicodynamic implications (Kim *et al*. 2006). As a result, and as discussed below, occupational exposures may underestimate the carcinogenic potential of benzene metabolites at low doses (5.3.1). Moreover, benzene-induced mortality is an irreversible frank effect mostly observed at occupational exposures but rarely observed at environmental concentrations. This does not represent a lack of carcinogenic events at low doses. Carcinogenesis is considered to involve toxicological events including clinical and preclinical that will lead to irreversible event – cancer mortality. Therefore, for a clearer understanding of the implications of benzene toxicokinetics, the toxicodynamic nature of benzene at low exposure concentration needs to be explored.

5.5.1 Mechanistic Understanding of Toxicodynamics

Toxicodynamic analysis should include the mechanistic understanding of the process of leukemogenesis, within a mode-of-action framework (Swenberg *et al*., 2008). In short, the framework should look for mechanistic pathways that lead to the pathogenesis of cancer (AML). The key events may involve genotoxicity, epigenetic events, and specific mutations related to the oncogenes. The choice of the appropriate quantitative method for dose-response analysis depends on the understanding of mode-of-action.

5.5.2 Mutagenic Mode-of-action

Genotoxic events implicated in the pathology of benzene include reverse mutation (e.g. Ames test – prokaryotic mutational screening tests), transversion, deletion, micronucleus formation, formation of DNA adducts, chromosomal strand breaks, and sister chromatid exchange (Section 3.5). However, the findings of various animal and human studies have not been consistent. Mutational studies have been performed in

cells of different species. Notably, results of reverse mutation assays with prokaryotic cells were not consistent. For example, *S. typhimurium* proved positive for histidine and azaquanine (Glatt *et al*., 1989, Kaden *et al*., 1979), and negative for the Ames test (De Flora *et al*., 1984). Gene mutation assays in prokaryotes or *in vitro* mammalian cell systems exposed to benzene and its metabolites revealed inconclusive results (Oberly *et al*., 1990; Glatt *et al*., 1989; Ashby *et al*., 1985; Oberly *et al*., 1984, Seixas *et al*., 1982; Kaden *et al*., 1979). By itself, inconsistencies in prokaryotic assays do not preclude a mutagenic component of benzene toxicity in humans. In fact, Nakayama *et al*. (2000) reported that the proportion of G:C → C:G transversion is significantly higher in human cells when compared to mouse cells (see Section 3.5) and suggested the possibility of different type of mutation in human cells (see Section 3.5).

Stakeholders points out a lack of specificity of covalent DNA adduct formation in target tissue of neoplasm has been suggested by Whysner *et al*. (2004) in rodents. However, Turteltaub and Mani (2003) have reported elevated DNA adduct formation of p-hydroquinone and t,t muconic acid in bone marrow in animals (section 3.8). This is in agreement with many of the human epidemiological studies involving benzene exposure which focus on the cellular events (e.g., neoplasia leading to leukomogenesis) in lymphocytes, including bone marrow (Section 3.8). In fact, DNA adducts and chromosomal aberrations were found consistently in bone marrow cells (i.e., the target cell) of benzene exposed individuals in an occupational setting (ATSDR, 2007). Indeed, the MOE concurs with the conclusion of ATSDR (2007) with respect to genetic effects, that no safe human exposure level can be determined from available epidemiological data.

As reviewed throughout this document, benzene concentration at low environmental exposure doses can produce certain genotoxic effects (e.g., micronucleus formation, DNA adducts formation, transversion, deletion, chromosomal strand breaks). However, stakeholders have raised concerns that no direct causative link between these molecular events and AML has been confirmed at benzene exposure concentrations typically found in urban ambient air in Ontario. MOE acknowledges that at present a causal-effect link has not been completely established directly between the molecular events and AML itself. However, molecular genetic events at the cellular level have been observed at low benzene exposure concentrations – just above typical urban background (e.g., increased micronucleus formation at 20 $\mu g/m^3$) – and these events are closely linked to the critical clinical end-point (e.g., AML see Section 3.8). Such DNA aberrations are further exacerbated by the inhibition of the topoisomerase II enzyme, which is required for repairing damaged DNA (see Section 3.1.3).

Based on the above discussions, the MOE concludes that mutagenic mode-of-action is a key component in the carcinogenesis of benzene.

5.5.3 Epigenetic Mode-of-action

Benzene and its metabolites also disrupt the cellular integrity through an epigenetic mode-of-action (Section 3.5; Section 3.8). Aneuploidy, DNA methylation, inhibition of topoisomerase II and oxidative stress are the major epigenetic pathways through which cellular damage is postulated to occur. Evidence of cellular effects at low doses of

benzene exposure is suggested from studies of aberrant DNA methylation patterns, including global hypomethylation, gene-specific hyper/hypomethylation and loss of genetic imprinting (Section 3.8). These are common in AML and other cancer tissues (Greiner *et al*., 2000; Galm *et al*., 2006; Bollati *et al*., 2007), and may represent an epigenetic component to the mode-of-action. The process of leukemogenesis, especially for AML, may involve many known and unknown pre-clinical events. As many of these epigenetic events may take place during the genesis of AML, MOE considers these epigenetic observations as potential pre-clinical events and as supporting evidence for the risk of AML at low dose exposure.

5.5.4 Hematological Mode-of-action

A number of stakeholders suggested that hematotoxicity may be a possible prelude to the genesis of AML. In such case, an observation of a hematological threshold may also imply a possible threshold in leukomogenesis. For example, Lan *et al*. (2004) discussed the possibility of hematological effects at a benzene concentration below 3200 $\mu g/m^3$ (1 ppm) (Section 3.8). However, Lamm and Grunwald (2006) expressed their concerns that there is no monotonical increase in WBC and granulocyte counts in the paper published by Lan *et al*. (2004) and suggest a possibility of hematological threshold. In reply to these comments, Lan *et al.* (2006) confirmed that the monotonicity of the association by spline regression analyses of WBC count and benzene exposure found no apparent threshold within the exposure range of the study [0.2 ppm (640 $\mu g/m^3$) to 75 ppm (240,000 $\mu g/m^3$)]. MOE agrees with the explanation of the Lan *et al.* (2006) and views this study as qualitative support for not identifying a threshold for benzene and blood cell effects.

As suggested by stakeholders, inconsistent hematological results at urban environmental human benzene exposure concentrations exist. The stakeholders might have considered clinical hematological events (e.g., decreased platelet count or other blood cell count) as the sole determinant of hematotoxicity. However, a decrease in blood cell count alone may not be a biomarker of effects for a possible mode-of-action for leukemogenesis. Rather, damage to the sub-cellular components in the hematopoietic system due to exposure of benzene (see Sections 3.1 and 3.8) may be an appropriate biomarker for a possible leukomogenesis link.

In summary, based on the toxicokinetic and toxicodynamic considerations (section 5.3, 5.4), benzene-induced leukemia (e.g. AML) is likely to involve multiple modes-of-action (i.e., mutagenic and epigenetic events) at the cellular level. However, which mode-of-action is predominant in leading the carcinogenesis as a result of benzene exposure is inconclusive.

5.6 Dose Response Analysis

Toxicological analysis of benzene (Section 3) does not identify a definitive threshold for the induction of AML, despite the suggestion by stakeholders of a “functional threshold” based on epidemiological findings. Hence the succeeding analysis will include only the non-threshold approach.

Linearized Multistage (LMS) model is one of the widely accepted models for carcinogenic risk analysis (US EPA, 1986). Currently, in its "Guidelines for Carcinogenic Risk Assessment", the Lowest Effective Dose (LED) model has been recommended by US EPA (2005). Linearized Multistage model (LMS) assumes occurrence of no effect in the absence of exposure. In the LED model, a range of observations is applied to a quantitative curve fitting model to generate a point-of-departure (POD). From the POD, if the chemical is mutagenic, a linear extrapolation model would be appropriate method. On the other hand, if the chemical is epigenetic a margin of exposure approach may be used.

5.6.1 Choice of Extrapolation Model for Low Dose Extrapolation

The important determinants for the selection of the model for carcinogenic dose-response analysis of benzene are modes-of-action criteria and the availability of the dose-effect data. The modes-action criteria include the mutagenic mode-of-action (see Section 5.4.2) and the epigenetic mode-of-action (see Section 5.4.3).

Cancer dose-response data can be obtained from animal or human epidemiological studies. In the case of benzene, the data sets from human epidemiological studies are preferred for a dose-response analysis over animal studies due to:

(i) Lack of reliable dose-response animal data with AML as the endpoint (Section 3.7).

(ii) Complexity of the metabolism and the lack of toxicokinetic data of the various metabolites preclude the validation of a reliable PBTK model for dose extrapolation from animals to humans (Section 3.7).

From the available human epidemiological studies, MOE considers Pliofilm data as the preferred data because (Section 3.7):

(i) The possibility of chemical interaction is low, since benzene was the only solvent used in Pliofilm industries as compared to most of the petrochemical studies

(ii) Exposure concentration estimates are reliable since personal monitoring data was used, and was validated by various risk assessment groups

(iii) Large cohort size with many years of follow-up of mortality data

(iv) Proper unexposed (not exposed in an industrial setting) controls were included in this cohort

(v) A causal relationship with AML has been demonstrated according to the Bradford Hill criteria of causation, which includes strength of casual effect association, consistency, specificity, temporality, dose-response relationship, coherence and analogy due to benzene exposure (Yardley-Jones and Gray, 2001; Descatha *et al*., 2005).

The US EPA and EU have selected the Pliofilm study as the basis of their cancer risk analysis (discussed throughout Sections 3 and 4). Other jurisdictions such as the WHO, RIVM, CalEPA have adopted the risk analysis of US EPA and EU.

Original data from Pliofilm study is not available to MOE to perform a dose-response using the LED analysis. In such a condition, MOE has considered to evaluate the US EPA and the EU cancer analyses as the basis for the development of an AAQC.

5.6.2 US EPA Approach (1998 / 2000)

US EPA identified the Crump analysis of the Pliofilm (Crump 1992; Crump 1994) as the most exhaustive approach in predicting the low dose extrapolation of risk of death due to AML (Section 4.1.2). Crump (1994) analyzed 94 risk estimates by considering different combinations of the following factors as presented below:

(1) Different disease endpoints (e.g., different types of leukemia),

(2) Additive or multiplicative models, for both linear and non-linear extrapolation

(3) Linear/non-linear dose-response relationships (e.g., sublinear, supralinear),

(4) Both cumulative and weighted exposure measurements (Rinsky *et al*., 1981; Crump and Allen, 1984; Rinsky *et al*., 1987; Paustenbach *et al*., 1992).

Crump (1994) analyzed the cancer dose-response relationship of the Pliofilm data using various methods. This includes the LMS and the quadratic models which provided the best fit of data (see Sections 3.7 and 5.5). The unit risks calculated based on the LMS method ranged from 2.2×10^{-6} to 7.8×10^{-6}, whereas the unit risks derived based on the quadratic model extrapolation ranged from 9.7×10^{-8} to 1.7×10^{-7}, both for a benzene exposure concentration of 1 ppb (3.2 µg/m^3). The choice of the LMS model by the US EPA (2000) is likely that it was the linear model available, given the LED model was adopted in 2005.

In general, the risk estimates would fall into the lower end (low risk) of the range if a sublinear exposure dose-response model was found to be more plausible. As demonstrated throughout this document, however, the shape of the exposure dose-response curve cannot be characterized without an understanding of the biological mechanisms of benzene-induced leukemia. In their assessment, the US EPA stated that an understanding of the mechanisms by which exposure to benzene and its metabolites exert their toxic and carcinogenic effects remained uncertain (US EPA, 1998). However as outlined above (see Section 3.8), current mechanistic evidence suggest that likely more than one mechanistic pathway may be responsible for the process of leukemogenesis. Yet, not enough information is available to accurately determine the specific shape of the dose-response curve at environmental exposure levels. Thus, it is not possible to provide a sound scientific basis to choose any particular extrapolation model to estimate human cancer risk at low doses over another. The US EPA supported the idea of a linear extrapolation because of the ability of benzene to cause damage to chromosomes even at low exposure concentrations. The US EPA further identified metabolic and molecular work conducted by a number of groups which lend suggestive support to the linear extrapolation (Irons *et al*., 1992; Irons and Neptune, 1980; Subrahmanyam *et al*., 1991; Eastmond, 1993; McDonald *et al.,* 1993; McDonald *et al.*, 1994; Pathak *et al*., 1995) (Section 4.1.2).

The linear model assumes a risk at any exposure – a key assumption in a mutagenic mode-of-action. Given the outlined uncertainties at low doses (see Section 3.8), the range of cancer unit risk estimates is narrowed down by a range between 7.1×10^{-3} and 2.5×10^{-2} at 1 ppm (2.2×10^{-6} to 7.8×10^{-6} at 1 μg/m^3 of benzene in air), depending on which exposure measurements were used (that is, Crump and Allen, 1984, or Paustenbach *et al*., 1992) (US EPA 1998, US EPA 2000). This range of risk estimates reflects both the inherent uncertainties in the risk assessment of benzene (e.g., mode of action of benzene toxicity and etiology of leukemia), and the limitations of the epidemiologic studies in determining dose-response and exposure data (see Section 3.7). However, one key assumption of this model is that below the point of departure linearity is assumed, despite that fact that there may be a sublinear component due to hypothesized epigenetic events early in leukomogenesis (see Section 5.4). Presence of such a phenomenon may represent a deficiency in the utilization of the linear approach (see Section 5.4). Conversely, recent data revealing potential benzene mode-of-action may suggest supralinear responses at low doses (see Section 5.3).

5.6.3 EU Approach (1998)

The EU used both the Pliofilm cohort study (Crump, 1994) and a meta-analysis of petrochemical cohort (Wong and Raabe, 1995) in their approach (EU, 1998) (see Section 4.1.3). The EU presented the argument that the linear extrapolation of the Pliofilm data (WHO, 1996, later updated in 2000) overestimated the results of the meta-analysis by Wong and Raabe (1995). Specifically, the comprehensive meta-analysis includes: data from more than 208,000 petrochemical workers, an estimated average exposure of 700 μg/m^3, and 148 deaths due to AML were identified (as compared to the expected 155 deaths due to AML) (Wong and Raabe, 1995) (see Section 4.1.3). In summary, EU heavily relies on the fact that the linear extrapolation of Pliofilm data should have resulted in high AML-related death in the petrochemical meta-analysis, however such results were not observed in the petrochemical cohorts (Wang and Raabe 1995). Therefore, the EU does not appear to support the linear model for the analysis of the Pliofilm cohort data. They felt that, linear extrapolation from occupational risk estimates, such as Pliofilm cohort is likely to produce a substantial over-estimation of the leukemia risk. The EU therefore considered that the petroleum chemical cohort data indicated a sublinear relationship between exposure and response. It is worth noting, however, that the EU did not develop a sublinear dose-response analysis. Rather, they commented on the results observed from the meta-analysis data of the petrochemical cohort when compared to the linear extrapolation of Pliofilm. The EU also acknowledged the fact that significant uncertainties exist in these epidemiological studies – some of which have been highlighted in this document (see Section 4.1.3).

As a result, the EU Panel acknowledged a range of 0.2-20 μg/m^3 as the guideline (Table 5.1), representative of a 10^{-6} risk value (see Section 4.1.3 for EU rationale). The low end of the risk-specific concentration of 0.2 μg/m^3 represents the WHO analysis based on the linear model of the Pliofilm data. The high end of the risk-specific concentration of 20 μg/m^3 represents the analysis of EU panel using the data of Wang and Raabe and Pliofilm cohorts.

In fact, EU concluded that the linear end may be any where from 0.2 to 20 μg/m^3. It is worth examining the meta-analysis of Wong and Raabe (1995) a little further. A number of points regarding EU interpretation of sublinearity and exposure estimates are highlighted below:

1. Wong and Raabe report an increase in leukemia following benzene exposure. The meta-SMR reveals that AML is less than 1, suggesting that benzene may not contribute to additional mortality due to AML at the exposure concentrations examined. Specifically, the observed meta-SMR for AML is 0.93 with 95% confidence limits of 0.73-1.16 (Table – 3.4). Thus, despite the large cohort study of Wang and Raabe (1995), the large variation in the confidence interval suggests that the apparently decreased SMR should not be over-interpreted.
2. A clear dose-response threshold has not been established in the Wong and Raabe data.
3. In the Wong and Raabe petrochemical cohort (see Sections 3.1.5 and 5.3), at high concentrations of benzene, toluene, ethyl benzene and xylenes, and saturation of CYP2E1 is likely to occur and will slow down the formation of the metabolites of benzene. In addition, the presence of toluene, ethyl benzene and xylenes will compete with benzene for CYP2E1; therefore further reducing the formation of the benzene metabolites. However, the metabolites of these petrochemicals other than benzene are not considered to be carcinogenic. These key events of saturation and chemical interaction may account for the lack of significant mortality observed in the cohort – possible under estimation of leukemia risk of benzene.

In summary, the cancer risk analyses of the US EPA and EU has their merits and limitations.

The US EPA (2000) considered a linear non-threshold low dose extrapolation model (LMS) as a default extrapolation approach for genotoxic carcinogens such as benzene, before the availability of new cancer risk assessment guidelines (US EPA, 2005) were adopted. The LMS method used by US EPA does not characterize either sublinearity or supralinearity of the dose-response model at low doses. Evaluation of the toxicokinetics and toxicodynamics (see Section 3) of benzene does not appear to identify a definitive threshold for benzene-induced AML risks. It is becoming increasingly clear that it may not be possible to ascertain the shape of the dose-response curve at low doses. Based on the toxicokinetic analysis of Rappaport *et al*., (2009), the possibility of a supralinear dose-response trend exists at doses as low as 3.2 μg/m^3, (see Section 3.8). The possibility of a sublinear dose-response trend is suggested by EU, based on the Wong and Raabe (1995) (see Section 5.5.3) analysis of the Pliofilm cohort. However, the toxicokinetic data at low doses (Kim et al., 2006; Rappaport *et al*., 2009; see Section 3.8) do not appear to support their view. In fact, the EU Panel presented a 0.2-20 μg/m^3 guideline range (Table 5.1), representative of a 10^{-6} risk value (see Section 4.1.3). Specifically, the EU Panel performed low dose linear extrapolation from the Pliofilm cohort data to arrive at the 0.2 μg/m^3 guideline value, to represent the low end of the range. However, the derivation of the inhalation unit risk for the high end value of 20

µg/m^3 is not available for validation. For this reason the range of risk-specific concentration of 0.2 to 20 µg/m^3 cannot be used as a basis for the derivation of AAQC of benzene. In addition, human epidemiological studies reveal chromosomal damages at benzene exposure concentrations at benzene exposure concentrations at the high end of the EU range (i.e., 20 µg/m^3 – Maffei *et al*., 2005 - Section 3.8 and Section 5.3). Therefore, it further lends support to the decision of the MOE not to consider the EU range of the risk-specific concentrations.

In the view of MOE, the shape of the dose-response curve at low dose cannot be characterized with confidence. The MOE evaluated the toxicokinetic analysis, mutagenic mode-of-action of the benzene metabolites, and the linear approach used by US EPA in their quantitative cancer risk analysis and supports the US EPA linear model as an appropriate method for low dose extrapolation for benzene cancer risk. Thus the MOE considers, the range of risk-specific concentrations of 0.13 to 0.45 µg/m3 from the US EPA for the derivation of an AAQC for benzene.

5.7 Low dose extrapolation

Mortality due to AML is an observation often associated with high industrial exposures. Both the Pliofilm study and the petrochemical meta-analysis by Wong and Raabe (1995) examined mortality due to leukemia resulting from benzene exposure. However, relying on mortalities may underestimate the risk because of possible cancer therapy, and a possibility of misclassification of leukemic types. Further, polymorphic genes of enzymes such as CYP2E1 and topoisomerase may predispose certain individuals to benzene toxicity (Kim *et al* 2006; Kim *et al* 2007). Thus prevalence of genetic polymorphisms may add uncertainty in the cancer risk assessment for benzene.

Patients who develop hematological abnormalities characteristically exhibit hypoplasia and pancytopenia. Aplastic anemia is usually seen while workers are still being exposed to benzene. In a substantial proportion of cases of benzene-induced anemias, acute myeloblastic leukemia, erythroleukemia or multiple myeloma developed during continued exposure to benzene (Rubin and Farber, 2000). Such bone marrow disorders may lead to other types of immunological effects which may lead to death, even without the initiation of cancer. The incidence of such events may add to the severity of total risk due to benzene exposure.

In summary, a linear dose-response in toxicokinetic and preclinical cellular events that have been postulated to play a role in the pathogenesis of AML, have been suggested from a number of critical toxicological events in both animals and humans. These events have been at exposure concentrations that ranged from less than 1 µg/m^3 to 1600 mg/m^3 (See Sections 3.8, 5.3, 5.4). For example:

(i) Toxicokinetic studies using DNA adduct formation from benzene metabolites as a biomarker, indicate a linear dose-response relationship in the range of 1.6 µg/m^3 to 1.6 mg/m^3 (Turteltaub and Mani, 2003)

(ii) Dose-dependent benzene metabolite-DNA adducts formation from as low as 0.7 µg/kg to 16 mg/kg of benzene in mice (0.97 µg/m^3 to 22

mg/m^3) (Creek *et al*., 1997) also suggested toxicokinetic linear dose-response relationship

(iii) The formation of DNA adducts by benzene metabolites in rat was linear at benzene concentrations from as low as 78 ng/kg (1.16 µg/m^3) (Mazzullo *et al*., 1989), to as high as 780 µg/kg (11.6 mg/m^3)

(iv) Formation of micronucleus at low dose exposure concentrations. (e.g., 20 µg/m^3 – Maffei *et al*. 2005)

(v) Irreparable genetic damage has been shown in humans exposed to benzene in a concentration range of 28 to 276 µg/m^3 (Bollati *et al*. 2007)

(vi) It has been hypothesized that DNA-repair mechanism interference may explain a statistically significant dose-response curve of DNA damage in benzene exposed workers, at benzene concentrations ranging from 4.45 µg/m^3 to 360 µg/m^3 (Chanvaivit *et al*., 2007)

(vii) Chromosomal aberrations were observed in humans exposed to benzene at a concentration range of 64 µg/m^3 to 1600 mg/m^3 (Zhang *et al*., 2002)

Considering the linear toxicokinetics in the low dose range and the possible preclinical toxicodynamic events that occur at low doses, the choice of linear low dose extrapolation is supported. Thus, the range of the inhalation unit risk (2.2 x 10^{-6} to 7.8 x 10^{-6}**)**, resulting from the linear extrapolation of the Pliofilm study is justified as the basis for the derivation of an AAQC (Crump, 1994 and US EPA, 2000).

5.8 Development of an Ontario Ambient Air Quality Criterion for Benzene

The "Guideline for the Implementation of Air Standards in Ontario" dated July 2005 (GIASO) outlines Ontario's risk-based decision making process for dealing with implementation issues related to updating air standards and air dispersion models, and described Ontario's air standard setting process (MOE, 2005).

In setting effects-based air standards, MOE considers the available toxicological information to determine the potential effects of exposure to a contaminant. The health risk for carcinogens is normally expressed as a "probability of occurrence". As demonstrated by Table 4.4 which compares the air quality guidelines and standards for various world-wide agencies, the scale of risks may range from a 1 in 10,000 risk (also expressed as 10^{-4}) (i.e., the risk of one individual in a population of 10,000 exposed developing some form of cancer) to a 1 in 1,000,000 risk (also expressed as 10^{-6}) (i.e., risk of one cancer in a population of one million). The MOE air standards objective for carcinogens is to set the standard at an incremental risk of 1 in a million (or 10^{-6}).

MOE understands that the mortality data from the occupational cohorts alone cannot support the choice of using either a linear or non-linear model for low dose risk

extrapolation. Hence, the shape of the dose-response curve at low dose cannot be characterized with confidence. The MOE evaluated the toxicokinetic analysis, modes-of-action (mutagenic and epigenetic) of the benzene metabolites, and the linear approach used by US EPA in its quantitative cancer risk analysis, along with the stakeholders' comments, and supports a linear model as an appropriate method for low dose extrapolation for benzene cancer risk. The Ministry has decided to select the range of inhalation unit risks of 2.2×10^{-6} to 7.8×10^{-6} by US EPA as a basis for the development of an AAQC. The corresponding risk specific concentrations of 0.13 to 0.45 µg/m^3, represents a life time additional cancer risk at a risk level of one in one million.

However, the Ministry considers that the high end of the range, i.e. 0.45 µg/m^3, may be more appropriate in the derivation of an AAQC for benzene due to the reasons discussed below. The US EPA linear extrapolation range of unit risk estimates (2.2×10^{-6} to 7.8×10^{-6}**)** were from Crump (1994). The low end of this range (2.2×10^{-6}) was calculated using the Paustenbach *et al*., (1992) exposure estimates of the Pliofilm study, whereas the high end of the range (7.8×10^{-6}) was calculated using the Crump and Allen (1994) exposure estimates of the Pliofilm study (Figure 3.8, Texas, 2007). Overall, the Paustenbach *et al* (1992) calculation of exposure matrix includes further follow-up on the Pliofilm (e.g. calculation of exposure matrix, and includes observation from Rinsky *et al*., 1987) when compared to Crump and Allen (1984) analysis (see Figure 3.8 and Section 4.1.2). Moreover, the best-fitting linear model for AML from Crump (1994) was based on weighted cumulative exposure matrix (Texas, 2007) – that is, the exposure matrix described by Paustenbach *et al*. (1992) (Texas, 2007). Hence, the unit risk of 2.2×10^{-6} calculated by Crump (1994) using the Paustenbach exposure matrix is recommended by MOE. The risk-specific concentration corresponding to one in one million is 0.45 µg/m^3.

5.9 Other considerations

5.9.1 Sensitive Sub-population - Children

There are a few scientific publications that are based on association of benzene exposure in the environment and prevalence of increased incidence of AML in children. One of the examples is the study by Whitworth *et al*. (2008). Childhood lymphohematic cancer incidences were analyzed with children exposed to air pollutants including benzene and 1,3-butadiene in southeast Texas during 1995-2004. Modeled benzene exposure concentrations in the range of 0.42 to 9.05 µg/m^3 were associated with non-statistical increase in AML incidence. However, co-exposure to 1, 3-butadiene may confound such findings. Hence, the authors could not confirm a clear association between benzene exposure and occurrence of AML in this study.

Cytochrome P450 is the rate limiting enzyme involved in the metabolic break down of benzene. This enzyme is less active at early stages of development; hence the chance of metabolic activation of benzene is greater in adults. A detailed explanation has been included in section 3.4. Hence, MOE considers the developing of AAQC for general public should also be protective of younger individuals.

5.9.2 Allocation to Other Sources of Exposure

Benzene is a systemic toxicant. The major route of exposure is through inhalation. However, benzene can be absorbed through other routes of exposure (e.g., ingestion – 3.1.1). Therefore, it is reasonable to examine if exposure from other sources (e.g., water, soil) may contribute to the total body burden of benzene.

Most regulatory agencies attribute more than or equal to 80% of benzene exposure to the air, which is a generic practice for most of the volatile organic compounds. The major reasons for more than 80% apportioning is based on that benzene is found at a greater abundant in the air than in any other media in terms of amount and concentration. Low water solubility of benzene is also a major criterion and the other physicochemical properties (e.g., high volatility) support the presence of benzene predominantly in air. In Ontario, the maximum benzene concentration in potable water was recorded as 0.35 µg/L (MOE – DWSP, 2009). This suggests that benzene from water may not be a major source of exposure in Ontario. Soil contamination does not lead directly to significant levels of human exposure to benzene, since benzene volatilizes rapidly from soil (IPCS, 1993). Benzene levels in the soil surrounding industrial facilities that produce or use benzene have been reported to range between < 2 and 191 µg/kg (U.S. EPA, 1979 and IARC, 1982). Health Canada (CEPA, 1996) reported inhalation as the major route in nation-wide benzene exposure data assessment. The exposure data resulted in a range of 70-98% through inhalation (e.g., indoor and ambient air, including mobile source). Other source contribution includes 0.7-2.7% from food and 0.6-4.5% from drinking water. Furthermore, the Federal Provincial Territorial Committee (FPTC) report on benzene in water concluded that 98-99% of benzene exposure occurs via air (FPTC, 2008).

As such, it does not appear to be reasonable to apportion the AAQC to routes of exposure other than inhalation.

6.0 Recommendation of Ambient Air Quality Standards for Benzene

Considering the significance and scientific information currently available, the cancer effects (e.g., AML) of benzene is critical. In consideration of the cellular effects of benzene on human health, the strength of the cancer risk assessment of other jurisdictions, followed by up to date scientific analysis, a unit risk factor (URF) of 2.2 E-07 per µg/m^3 derived by linear extrapolation of the Pliofilm cohort data is determined to be scientifically justified for the development of the AAQCs and air quality standards for Benzene. From this URF, the lifetime excess cancer risk-specific concentration of 0.45 µg/m^3, at the one in one million risk level, is derived.

Thus the Ministry proposes the following AAQCs and air quality standards for Benzene:

Proposed Annual Average AAQC for Benzene:

- **An annual average Ambient Air Quality Criterion (AAQC) of 0.45 µg/m^3 (micrograms per cubic metre of air) for Benzene, based on carcinogenicity associated with exposure to Benzene,**

Proposed 24-hour Average AAQC for Benzene:

- **A 24-hour average Ambient Air Quality Criterion (AAQC) of 2.3 µg/m^3, (micrograms per cubic metre of air) for Benzene, based on carcinogenicity associated with exposure to Benzene,**

Proposed 24-hour Average Standard for Benzene:

- **A 24-hour average standard of 2.3 µg/m^3 (micrograms per cubic metre of air) for Benzene, based on carcinogenicity associated with exposure to Benzene, and**

Proposed ½-hour Average Standard for Benzene:

- **A half-hour average standard of 7 µg/m^3 (micrograms per cubic metre of air) for Benzene, based on carcinogenicity associated with exposure to benzene.**

After consultation on these proposed standards, the Ministry's intent is to arrive at a decision regarding the effects-based AAQCs and the corresponding effects-based 24-hour and half hour standards. The AAQCs will be added to Ontario's Ambient Air Quality Criteria and the 24-hour standard and half-hour standard will be incorporated into schedule 3 and Schedule 2 of Regulation 419/05, respectively.

MOE generally proposes a phase-in for new standards or standards that will be more stringent than the current standard or guideline. The phase-in for benzene is set out in O.Reg. 419/05.

Among other things, O.Reg 419/05 sets out the applicability of standards, appropriate averaging times, phase-in periods, types of air dispersion models and when various sectors are to use these models. There are 3 guidelines that support O.Reg 419/05. These guidelines are:

- "Guideline for the Implementation of Air Standards in Ontario" (GIASO);
- "Air Dispersion Modelling Guideline for Ontario" (ADMGO); and
- "Procedure for Preparing an Emission Summary and Dispersion Modelling Report" (ESDM Procedure).

GIASO outlines a risk-based decision making process to set site specific altered air standards to deal with implementation barriers (time, technology and economics) associated with the introduction of new/updated/air standards and new models. The altered standard setting process is set out in Section 32 of O.Reg. 419/05.

For further information on these guidelines and O.Reg. 419/05, please see the Ministry's website at http://www.ene.gov.on.ca/ and follow the links to local air quality.

7.0 A Guide for Stakeholders Reviewing this Rationale Document

The Ministry welcomes written comments on this rationale Document from all interested parties. Stakeholders are encouraged to provide comments which indicate whether they support or disagree with the above recommendations. It is also important that submissions include the rationale and reasoning supporting the stated positions so that the Ministry can make informed decisions on the proposed standard on the basis of clear, supportable arguments.

Comments on these and any other issues relevant to setting of air quality standards for benzene can be sent to:

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9.0 Acronyms

CalEPA	California Environmental Protection Agency
CAS	Chemical Abstracts Service
HC	Health Canada
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
RIVM	Dutch National Institute for Public Health and the Environment
TC_{05}	tumourigenic concentration which induces a 5% increase adverse effects
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

10.0 Abbreviations

^{o}C	degrees in Celsius
bw	body weight
g	gram
mg	milligram, one thousandth of a gram
µg	microgram, one millionth of a gram
ng	nanogram, one billionth of a gram
ppm	parts per million
ppb	parts per billion

11.0 Definitions

Acute erythroleukemia – It is a rare entity comprising of 5 to 6% of cases of AML. Rapid proliferation of immature erythroid precursor (≥80%) with no significant myeloblastic component.

Acute lymphocytic leukemia – Rapid proliferation of immature lymphocytes, a type of white blood cell produced in lymph system and marrow

Acute myelogenous/myeloid leukemia (AML) – A malignant clonal proliferation of immature myeloid hematopoietic progenitor cells (i.e. myeloblasts), which are precursors to myeloid white blood cells; a neoplastic disease of the hematopoietic system in which there is diminished production of normal erythrocytes, granulocytes and platelets, which leads to death by anemia, infection or hemorrhage.

Acute myelomonocytic leukemia – It is a type of paediatric AML. Presence of more than 20% to 30% of myeloblasts in the bone marrow with more than 20% of non-erythroid cells of monocytic origin

Acute promyelocytic leukemia – It is a type of AML and is due to a translocation between chromosomes 15 and 17. It accounts for 5-10% cases of acute myeloid leukemia (AML) with mostly occurring in young adults.

Acute undifferentiated leukemia – Acute myelogenous leukemia in which the predominating cell is very immature and a classification is not possible

Chronic lymphocytic leukemia – A clonal expression of mature lymphocytes (i.e. B and T cells) in the peripheral blood and the bone marrow

Chronic myelogenous leukemia – A clonal expansion of pluripotent hematopoietic stem cells which do not mature normally. The clinical expression is gradual and the event progression is gradual.

Cytopenia – A decrease in various cellular elements of the circulating blood which is a common clinical finding in benzene hematotoxicity and may manifest itself as pancytopenia and aplastic anemia or as unicellular cytopenia (e.g. leukopenia, anemia, thrombocytopenia)

Dyscrasias – Dyscrasias is a non-specific term that refers to any disease or disorder. However, it usually refers to blood diseases.

Dyserythropoiesis – Any defect of RBC production characterized by morphologic abnormalities of the nuclei and cytoplasm, which may be acquired

Dysgranulopoiesis – Non-neoplastic condition that may mimic true Myelodysplasia (an AML condition) e.g. Neutrophil hypogranularity is form of dysgranulopoiesis.

Dysmegakaryopoiesis – Non-neoplastic condition that may mimic true Myelodysplasia (an AML condition). Manifest as megakaryocytes with separate round hypolobated nuclei

Hairy cell leukemia – It is a rare proliferation of lymphocytes to leukemic condition and the cells develop microscopic hair like structure.

Hodgkin's lymphoma – A malignant solid tumor of the lymphoreticular system, usually the lymph nodes, diagnosed by the presence of the Reed-Sternberg cell.

Hyperplasia – Increased cell production in a normal tissue or an organ. Hyperplasia may be a sign of precancerous change.

Hypocellular acute leukemia – Increase of blasts (immature cells), > 20% in the bone marrow and/or in the peripheral blood, with displacement of normal hematopoietic elements, resulting in hypocellular bone marrow.

Lymphosarcoma – Malignant tumour of lymphatic tissue

Multiple myeloma – It is a type of cancer that begins in plasma cells (white blood cells that produce antibodies) thus affecting the immune system.

Pancytopenia – A shortage of all types of blood cells, including red and white blood cells and platelets.

Physiologically based toxicokinetic model (PBTK) – Mathematical description of the toxicokinetic turn over (Absorption, Distribution, Metabolism and Elimination) of toxics in a biological system (e.g. human or mammals) considering the physiological principles (e.g. blood flow rate, blood tissue partition co-efficient, metabolic rate constants, etc.).

Preleukemia – A group of nondiagnostic physical and blood abnormalities that may indicate that leukemia may develop later. They include: anemia, neutropenia, sometimes a relative lymphopenia, marked monocytosis, purpura, susceptibility to infections, and slow healing of skin and mucous membrane lesion. Signs include loss of leukocytes and other blood elements, bone marrow histopathology, and enlarged spleen. Myelodysplastic syndromes are preleukemic

Reticulum cell sarcoma – A malignant tumor of reticular tissue that is composed primarily of neoplastic histocytes

RR – Relative risk (or risk ratio). Measures the number of times an adverse health event occurs relative to the total number of people in the study

SMR – Standardized mortality ratio. A statistical tool; a standardized event ratio used for comparing mortality observed in an exposed population to the mortality observed in a standard population.

Toxicokinetics – The fate of a chemical exposed to a biological system (e.g. humans) which includes absorption, distribution, metabolism and elimination (The way the body handles the chemical).

Toxicodynamics – The effect of a chemical on the biological system (e.g. humans) in causing damage to the system (The impact of the chemical on the body).

Zymbal gland carcinoma – Cancerous tumour originating from an auditory sebaceous (fatty) gland that opens into each external ear canal known as Zymbal's gland.

12.0 Appendix A: PBTK Data and Models - Background

The PBTK approach seeks to predict the dose-response characteristics of a chemical's potential toxicity. It does this through an understanding of the underlying mechanisms by which a substance is absorbed, transported to, and metabolized in the primary target organs, on the assumption that the biochemical processes involved in the toxicological response will be the same for both humans and the animal model(s) in which the harmful effects of the compound had been demonstrated. One of the major advantages of these models is the possibility of high to low dose extrapolation along with proper validation. Necessary inputs to the model include a physiologically realistic discrimination of a chemical's movement between metabolically and functionally linked compartments of the body and the information necessary to describe this movement mathematically in terms of

(1) The kinetics of metabolism,

(2) Rates of movement to and from different target organ groupings, and

(3) The partitioning of a compound between physiological media.

Thus, by modeling a target organ-specific internal dose surrogate (parent compound or metabolite, as applicable) from the toxic dose of an experimental study, an equivalent allometrically scaled internal dose surrogate in human beings can be used to back-extrapolate to a hypothetical "effective" dose for the same toxicological response in human beings. A schematic representation of PBTK can be seen in figure 3.4. A four compartment model has been schematically described. The representation of lung and kidney in the figure 3.4 is mainly to illustrate the inhalation and the excretion pathways and has not been included as a compartment in benzene PBTK model simulation.

The first model for benzene was developed by Sato and co-workers (Sato and Nakajima, 1979; Sato, 1988), who exposed three men to 25 and 100 ppm (80 and 319 mg/m^3) benzene vapour for 2 hours and then observed a tri-exponential decay of benzene from their blood. The investigators constructed a three-compartment model consisting of richly perfused tissues, poorly perfused tissues, and fat, which acted as a major sink for benzene. Since then several attempts have been made in the development of PBTK models involving different level of complexity and several compartments as well. Subsequently, PBTK models have been developed to take into account differences in benzene and its metabolites between species and individuals using both experimental data and simulations (Medinsky *et al*., 1989; Travis *et al*., 1990a; Travis *et al*., 1990b; Bois *et al*., 1991; Cox, 1991; Cox, 1996). Following oral exposure, rats metabolized more benzene on a body-weight basis than did mice at doses greater than 50 mg/kg (Medinsky *et al*., 1989a; Medinsky *et al*., 1989b; Medinsky *et al*., 1989c). Patterns of metabolites also differed between rats and mice. Mice produced primarily hydroquinone glucuronide and MA metabolites linked to toxic effects; on the other hand, rats produced primarily phenyl sulfate, a detoxification product. These simulated results agree with experimental data and provide a framework for understanding the greater sensitivity of the mouse to benzene toxicity.

The Medinsky model was based on an earlier PBTK model developed by Ramsey and Anderson (1984). Metabolism of benzene, consisting of initial metabolism to benzene oxide that is then further metabolized by one of four pathways, was modeled by Michaelis-Menton kinetic parameters (Km – Michaelis-Menton constant; and V_{max} – maximum velocity of the forward reaction). Metabolic rate constants were determined by fitting the results of model simulations to experimental data obtained by exposing mice and rats to benzene orally and by inhalation (Medinsky *et al*., 1989b; Sabourin *et al*., 1987). However, Bois *et al*. (1991b) found that the Medinsky model did not simulate the data of Rickert *et al*. (1979) very well, thereby indicating a need for the model to be further refined.

Since the original Medinsky model was published, additional compartments have been added to reflect an advancing understanding of benzene metabolism, and specific biochemical and toxicokinetic parameters have been refined to reflect age, sex, and species-specific differences (Schlosser *et al*., 1993; Seaton *et al*., 1994; McMahon *et al*., 1994; Kenyon *et al*., 1995). Seaton *et al*. (1994) measured a 13-fold variability in CYP2E1 activity in human hepatic microsomes and compared this to the activity in mouse and rat liver microsomes. The model predicted the dependence of benzene metabolism on the measured CYP2E1 activity, and the proportion of hydroquinone (the suspected toxic metabolite) produced in vitro was correlated with the level of CYP2E1 activity. Seaton *et al*. (1995) measured the initial rates of the two major conjugation reactions, phenol sulfonation and hydroquinone glucuronidation, in the hepatic microsome preparations of humans, rats, and mice. This information was used in a physiological compartment model to predict steady-state concentrations of phenol and hydroquinone in blood.

The predicted steady-state concentration of phenol and hydroquinone concentrations varied in different animal species and in human. On this basis, the authors suggested that the rat may be a good model for humans with respect to tissue dosimetry for these benzene metabolites. The authors also suggested that the mouse might be more sensitive than the human and that *in vitro* metabolism data must always be placed within the context of the whole animal's physiology.

The Medinsky PBTK model has served to organize the available information into a coherent model that has helped to refine the specific experimental approaches used to fill the gaps in the understanding of the mechanism of benzene toxicity. Although current PBTK models may provide insights about putative toxic metabolites and potential biochemical mechanisms, they are insufficiently developed to be able to reduce scientific uncertainty (Medinsky *et al*., 1995, 1996; Medinsky, 1995).

Travis *et al*. (Travis *et al*.1990 a; Travis *et al*.1990 b) also developed a model to describe the pharmacokinetics of benzene in rats, mice, and humans. The model contains five compartments, consisting of liver, fat, bone marrow, muscle, and organs (such as brain, heart, kidney, and viscera). The different compartments are connected by the arterial and venous blood pathways. Metabolism of benzene is assumed to follow Michaelis-Menton kinetics in all species and is assumed to occur primarily in the liver and to a lesser extent in the bone marrow. Model simulations were compared with experimental data from Sabourin et al. (1987, 1988a), Andrews *et al*. (1977, 1979),

Nomiyama and Nomiyama (1974), Snyder *et al*. (1981), Sato *et al*. (1975), and Rickert *et al*. (1979). The Travis model successfully simulated uptake, metabolism, and excretion of benzene for mice, rats, and humans using experimental data from the studies that were used to develop the model. However, the model is of limited value because it does not predict the kinetics of benzene metabolites (Bois *et al*., 1991b).

The model developed by Bois and Paxman (1992) provided evidence that exposure rate had a strong influence on the rate of formation of several important metabolites of benzene. This model has three components describing the pharmacokinetics of benzene and the formation of metabolites in the rat. The model was validated against the data of Cassidy and Houston (1984), Sabourin *et al*. (1987, 1988a, 1989), and Sawahata and Neal (1983). It was also used to predict metabolite production for male rats exposed to benzene (nose only) at three different concentrations and for three different exposure durations in comparison with the experimental data of Sabourin *et al*. (1989). The three exposure regimens were established to maintain a constant concentration/time product. Simulation results indicated that the model may over- or underestimate the level of urinary metabolites.

More recent efforts on development of the Bois and Paxman model have focused on defining the PBTK parameter distributions needed to develop models useful in risk assessment (Spear and Bois 1994; Spear *et al*., 1991; Watanabe and Bois, 1996; Bois *et al*., 1991a, 1996). Spear and Bois (1994) described the outcome of their modeling efforts to explain the basis for the paradoxical observation that although phenol is a major initial metabolite of benzene, a known carcinogen, a National Cancer Institute chronic study (NCI, 1980) did not demonstrate carcinogenic activity for phenol. The approach selected was to apply Monte Carlo methods using parameter distributions coupled with a pass-fail fit criterion. The advantage of this approach is that it acknowledges that in most biological applications, there is no clear way to select a "best" set of fixed parameters. On the basis of the researchers' modeling effort, hydroquinone was rejected as the ultimate toxic agent, and the pathway through benzene glycol to catechol and MUC appeared to provide a better fit to the data.

Kenyon *et al*. (1995) investigated the metabolism of phenol. Even though phenol is thought to be a key intermediate in benzene metabolism leading to toxicity, orally administered phenol is neither carcinogenic or genotoxic (NCI, 1980). The authors found markedly higher excretion of hydroquinone glucuronide after oral benzene exposure as compared with phenol. Also, phenol sulfate and phenol glucuronide excretion was much lower following benzene exposure than following phenol exposure. This could be explained by differences in the zonal distribution of CYP2E1 and detoxification enzymes in the liver. Phenol initially entering the liver had a relatively greater chance for conjugation (sulfonation or glucuronidation) in periportal hepatocytes of zone 1 than of oxidation by CYP2E1 located in pericentral hepatocytes in zone 3. Benzene, on the other hand, was more likely to pass through to zone 3 and be oxidized to phenol.

Bois *et al*. (1996) applied techniques from population pharmacokinetics, Bayesian statistical inference, and physiological modeling to model distribution and metabolism in humans. Statistical distributions for the parameters of a physiological model of benzene were derived on the basis of existing data. The relationship between the fraction of benzene metabolized in bone marrow and benzene exposure was linear up to 10 ppm (32 mg/m^3). The median population estimate of the fraction metabolized in bone marrow was 52% (90% confidence interval [CI] 47–67%). At levels approaching occupational inhalation exposure (continuous 1 ppm [3.2 mg/m^3]), the estimated amount metabolized in bone marrow ranged from 2 to 40 mg/day. However, this model has not been tested for its ability to predict data from other studies (Smith and Fanning, 1997).

Indeed, the current published models are insufficiently refined to allow the prediction of human metabolism accurately; this is further complicated due to the advent of the complex low dose benzene metabolism events, explained in more detail in session 5. Krishnan and Andersen (2000) has compiled the later development of models with benzene. Further development with benzene with QSAR has also been put forth by different researchers. The key areas for refinements appear to be the inclusion of the kinetics of the putative toxic metabolites of benzene or their stable precursors. If benzene metabolites such as hydroquinone/ benzoquinone, MUC, and/or benzene oxide are the toxic species, then PBTK models need to include descriptions of their kinetics if they are to be useful in improving uncertainties in risk assessment. Below, a benzene PBTK model has been reconstructed by MOE for the purpose stated in the introducing paragraph of this chapter (p.44) using the parameters published by Haddad *et al*. (2001), and is discussed below.

PBTK for benzene – MOE Approach

Overall, physiologically based toxicokinetic model are mathematical representation of the chemical's toxicokinetic fate in a biological system along with the rules of animal/human physiology. They can be either deterministic or stochastic depending on the extent of parametric knowledge or its application. In our excise a deterministic PBTK model has been used. The parameters and the out come of the model along with a basic discussion on its application has been hi-lighted in chapter 4, however this appendix has been included to guide the readers through the mathematical part of this model (Table A.1).

Mathematical Representation:

In PBTK modeling, each tissue compartment is generally described with a mass balance differential equation (MBDE) that consists of a series of clearance terms with units of volume per time, i.e., liters per hour or milliliters per minute. The clearance terms, in most cases, relate to tissue uptake, tissue-to-blood transfer, metabolism, or excretion of chemicals. The uptake of chemical in systemic circulation by a tissue is described according to Fick's law of simple diffusion, which states that the flux of a chemical is proportional to its concentration gradient. Descriptions of passive and blood flow-limited uptake have been used successfully in many of the past effort in PBTK modeling that dealt with small molecular weight organic chemicals.

Computer Implementation:

The PBTK model requires the use of numerical simulation methods because they contain differential equations and description of non-linear processes. Therefore, the PBTK model equations are written along with the integration algorithms and solved using programming languages, simulation software, or spreadsheets. Programming language (Microsoft Excel®) has been used to simulate the benzene toxicokinetic profile and the model codes have been written for appropriate numerical integration algorithm. Euler, Gear and Runge-Kutta routines were used along with specific integration intervals and units.

Table A-1: PBTK model equations and their expression in Excel® spreadsheet (Haddad *et al*. 1996), as used in the present study for simulating toxicokinetics of benzene.

Compartment	Equation[a]	Expression in Excel®
Arterial blood	$Ca_n = \frac{Qc_n \times Cv_{n-1} + Qp_n \times Cinh_n}{Qc_n + Qp_n / Pb}$	$K20 = \frac{E20 \times AB19 + D20 \times J20}{E20 + D20/Pb}$
Liver	$dA_l / dt_n = Q_{ln} \times (Ca_n - Cv_{ln-1}) - \frac{V_{max} \times Cv_{ln-1}}{km + Cv_{ln-1}}$	$L20 = F20 \times (K20 - O19) - \frac{V_{max} \times O19}{km + O19}$
	$A_{ln} = dA_l / dt_n \times t + A_{ln-1}$	$M20 = L20 \times t + M19$
	$C_{ln} = A_{ln} / V_l$	$N20 = M20 / V_l$
	$Cv_{ln} = C_{ln} / P_l$	$O20 = N20 / P_l$
Fat	$dA_f / dt_n = Q_{fn} \times (Ca_n - Cv_{fn-1})$	$P20 = G20 \times (K20 - S19)$
	$A_{fn} = dA_f / dt_n \times t + A_{fn-1}$	$Q20 = P20 \times t + Q19$
	$C_{fn} = A_{fn} / V_f$	$R20 = Q20 / V_f$
	$Cv_{fn} = Cf_n / Pf$	$S20 = R20 / P_f$
Richly perfused tissues	$dA_r / dt_n = Q_{rn} \times (Ca_n - Cv_{rn-1})$	$T20 = H20 \times (K20 - W19)$
	$A_{rn} = dA_r / dt_n \times t + A_{rn-1}$	$U20 = T20 \times t + U19$
	$C_{rn} = A_{rn} / V_r$	$V20 = U20 / V_r$
	$Cv_{rn} = Cr_n / \mathrm{Pr}$	$W20 = V20 / \mathrm{Pr}$
Slowly perfused tissues	$dA_s / dt_n = Q_{sn} \times (Ca_n - Cv_{sn-1})$	$X20 = I20 \times (K20 - AA19)$
	$A_{sn} = dA_s / dt_n \times t + A_{sn-1}$	$Y20 = X20 \times t + Y19$
	$C_{sn} = A_{sn} / V_s$	$Z20 = Y20 / V_s$
	$Cv_{sn} = Cs_n / P_s$	$AA20 = Z20 / P_s$
Venous blood	$Cv_n = \frac{Q_{ln} \times Cv_{ln} + Q_{fn} \times Cv_{fn} + Q_{rn} \times Cv_{rn} + Q_{sn} \times Cv_{sn}}{Q_{cn}}$	$AB20 = \frac{F20 \times O20 + G20 \times S20 + H20 \times W20 + I20}{E20}$
	$Calv_n\,(mg/L) = Ca_n / Pb$	$AD20 = K20 / \mathrm{Pb}$
Alveolar air	$Calv_n(ppm) = Calv_n \times 24450 / MW$	$AE20 = AD20 \times 24450 / MW$

Knowledge of chemical specific species specific parameters is required to solve the MBDE constituting the PBTK model. For a model to be used for estimating interindividual differences in tissue dosimetry, knowledge of the distributions of input parameters is essential. However, for all other purposes the knowledge of the average value or the range of plausible values of model parameters is sufficient. Typically PBTK models require physiological, physicochemical and biochemical parameters. The values for benzene have been tabulated in Table A.2 and Table A.3.

Physiological Parameters:

In PBTK models for organic chemicals, the sum total of the volumes of compartments corresponding to soft tissues should be smaller than the body weight, usually about 91% of the body weight (9% weight of skeletal/structural components). Even though the tissue volumes (L) are needed for PBTK modeling, tissue weights (kg) are usually used with the assumption of unit density (L = kg). This assumption, which may seem questionable, is inconsequential for practical reasons, particularly with respect to the application of PBTK models in developing a toxicological reference value. The tissue flow rates in the model should add up to cardiac output. Fundamentally, maintaining the mass balance in PBTK model requires that the sum of the flows to the compartments be equal to the cardiac output. Specifically on deterministic models, ventilation rate, cardiac output, and tissue perfusion rates and tissue volumes are specified for an individual animal or human being simulated. An acceptable PBTK model should contain tissue volumes, flow rates, and ventilation: perfusion ratios that are within physiological limits. Particularly, the sum total of the tissue volumes should not exceed the body weight, and the sum total of tissue blood flow rate should equal cardiac output.

Table A-2: Physiological Parameters Used for Benzene-PBTK simulation

Parameters	Human Values
Alveolar ventilation rate (l/h/kg)	6.00
Cardiac output (l/h/kg)	5.31
Blood flow rate (fraction of cardiac output)	
Fat	0.05
Slowly perfused tissues	0.25
Richly perfused tissues	0.44
Liver	0.26
Volume (fraction of body weight)	
Fat	0.19
Slowly perfused tissues	0.62
Richly perfused tissues	0.05
Liver	0.026

Table A-3: Physicochemical and Biochemical Parameters used in PBTK Modeling of Benzene-Human

Parameters	Values
Blood: air	7.4
Fat: air	406
SPT: air	15.0
RPT: air	11.0
Liver: air	11.0
Vmax (mg/h/kg)	2.11
Km (mg/L)	0.10

Appendix B: Hematological data from Lan *et al*. (2004) resulting in suppression of referral blood cell count due to benzene exposure.

Table B: Peripheral blood cell counts in relation to benzene exposure.

Subject category (n)	Control (140)	Low dose (109)	Medium dose (110)	High dose (31)
Benzene air level (mg/m^3)	< 0.128	1.82	9.12	91.94
WBC	6480	5540	5660	4770
Granulocytes	4110	3360	3480	2790
Lymphocytes	2130	1960	1960	1800
CD4+-T cells	742	635	623	576
CD8+-T cells	553	543	564	549
B-cells	218	186	170	140
Monocytes	241	217	224	179
Platelets	230	214	200	172

Aadopted from Lan *et al*., 2004