Accepted Manuscript

Use of toxicant sensitivity distributions (TSD) for development of exposure guidelines for risk to human health from benzene

Benjamin Edokpolo, Qiming Jimmy Yu, Des Connell

PII: S0269-7491(19)31093-0

DOI: <https://doi.org/10.1016/j.envpol.2019.04.001>

Reference: ENPO 12401

To appear in: *Environmental Pollution*

Received Date: 27 February 2019

Revised Date: 29 March 2019

Accepted Date: 1 April 2019

Please cite this article as: Edokpolo, B., Yu, Q.J., Connell, D., Use of toxicant sensitivity distributions (TSD) for development of exposure guidelines for risk to human health from benzene, *Environmental Pollution* (2019), doi: <https://doi.org/10.1016/j.envpol.2019.04.001>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Development of Guideline Value from Toxicity Sensitivity Distribution Plot

**USE OF TOXICANT SENSITIVITY DISTRIBUTIONS (TSD) FOR DEVELOPMENT
OF EXPOSURE GUIDELINES FOR RISK TO HUMAN HEALTH FROM BENZENE**

Benjamin Edokpolo¹, Qiming Jimmy Yu¹ and Des Connell²

¹School of Engineering and Built Environment, Griffith University, Nathan Campus, Brisbane,
4111, QLD, Australia

Telephone +61 (0)7 3735 5289, Mobile +61 (0)438 173 802, Email jimmy.yu@griffith.edu.au

²School of Environment and Sciences, Griffith University, Nathan Campus, Brisbane, 4111, QLD,
Australia

ABSTRACT

This technique for setting guideline values differs from that currently used by regulatory agencies throughout the world. Data for benzene were evaluated from epidemiological studies on human populations (29 studies). Exposure durations were evaluated in terms of Long Term Exposure (LTE) and Lifetime Exposure. All data was reported as Lowest Observed Adverse Effect Levels (LOAEL) and converted into exposure doses using Average Daily Dose (ADD) and Lifetime Average Daily Dose (LADD). These values were plotted as a Toxicant Sensitivity Distribution (TSD) which was the cumulative probability of LOAEL-ADD and LOAEL-LADD. From the TSD plots, linear regression equations gave correlation coefficients (R^2) ranging from 0.69 to 0.97 indicating normal distributions. Guideline Values (GVs) for LTE (8hr/day) and Lifetime (24hr/70yrs) exposure to benzene were calculated using data from human epidemiological studies as 5% level of cumulative probability (CP) of LOAEL-ADD and LOAEL-LADD from the cumulative probability distributions (CPD). The derived guideline values from the human epidemiological studies were 92 μ g/kg/day for LTE and 3.4 μ g/kg/day for lifetime exposure. GV for LTE is appropriate for occupational exposure and GV derived for lifetime exposure appropriate for the general population. The guideline value for occupational exposure limit was below all the guideline values developed by regulatory agencies. But the general population guideline is within the range of values formulated by European Union, ATSDR, EPAQS, USEPA and OEHHA for air quality for the general population. This is an alternative method which eliminates the application of safety factors and other sources of errors in deriving guideline values for benzene.

Capsule:

An alternative technique of LOAEL based on toxicant sensitivity distribution curve was used to set guideline values for exposure to benzene as 92 μ g/kg/day for long term exposure and 3.4 μ g/kg/day for lifetime exposure.

Keywords: benzene, lowest observed adverse effects level, human epidemiological studies, long term exposure, lifetime exposure, guideline values.

1.0 INTRODUCTION

Benzene is used by many industries as an industrial solvent, an intermediate in chemical syntheses and it is a component of petrol. Exposure to benzene is mainly through inhalation although oral and dermal routes are other possibilities (ATSDR 2007). Exposure to benzene can occur in both indoor and outdoor settings with emissions from cigarette smoke being considered as one of the main indoor sources (Singer et al. 2003). Other indoor sources are stoves, wood heaters, fireplaces, solvent products, polystyrene, paints and vinyl floor coverings (Raw et al. 2004; Weisel et al. 2008). Major sources of outdoor exposure for the general population are tobacco smoke, service stations, emissions from exhaust of motor vehicles and industrial emissions. Industrial sources include petrochemicals, petroleum refining, storage or transport of benzene, processing, rubber tire manufacturing and coke and coal chemical manufacturing.

Exposure to benzene can cause a wide variety of adverse health effects as highlighted in various laboratory and epidemiological studies (Lan et al. 2004). Hematotoxicity has been consistently reported in humans and experimental animals exposed to benzene with bone marrow as the principal target organ. It has also been shown to produce neurotoxic effects in test animals and humans after short-term exposure. There is some evidence of reproductive and developmental effects due from human epidemiological studies. Benzene is a known human carcinogen through all routes of exposure and is rated as a major priority in protection of public from chemical exposure USEPA (2012a) and IARC (2010). Establishment of accurate guideline values for protection of human populations from exposure is a high priority.

Health risk assessment provides us with a systematic approach for characterising the nature and magnitude of the risks associated with environmental health hazards (USEPA 2002; USEPA 2005; enHealth 2012). Exposure to benzene can be evaluated using guidelines based on the USEPA Acceptable Daily Intake (ADI), Slope Factor (SF), Reference Dose (RfD) and ATSDR Minimal Risk Level (MRL) as reviewed by USEPA (2012a). WHO (WHO, 2010) and many other agencies have established guidelines and IACR (IACR, 2012) has concluded that benzene is a human carcinogen in Group 1. Many different approaches have been used including data on occupational exposure, experiments on surrogate animals, haematological effects on workers in addition the No Observed Adverse Effect Levels (NOAEL) and LOAEL were used with a variety of Safety or Uncertainty Factors. Thus, a range of guideline values are available to conduct risk assessments giving various evaluations of the hazard.

However, the probabilistic risk assessment technique is considered to give a better understanding of the risks by use of cumulative probability plots for the exposure and adverse effect data. The probabilistic techniques have been used extensively in our previous studies to evaluate health risk. Djohan et al. (2007) used probabilistic techniques to evaluate the health risk associated with exposure to chlorobenzene in the air of residential houses. Also, Hamindin et al. (2008) used probabilistic techniques in evaluating the health risk of chlorination disinfection by-products in drinking water. In a more recent study, the probabilistic technique was used in evaluating the risk for exposure to benzene in service station environments by Edokpolo et al. (2014). We have also developed new techniques for quantitative health risk assessment, particularly with the Overall Risk Probability (ORP) method (Cao et al. 2011; Yu et al. 2012) which was used in the studies of Phung et al. (2012) and Edokpolo et al. (2014 and 2015).

A new probabilistic method for setting guidelines to protect human health as a result of the use of chlorpyrifos in agriculture has been developed by Phung et al. (2015). A probabilistic dose – adverse response relationship, a cumulative probability distribution which can be described as a Toxicant Sensitivity Distribution (TSD), was established from published data available in epidemiological studies associated with exposure to chlorpyrifos. This was used to establish a guideline value for chlorpyrifos. In later publications, Phung (2016, 2017) expanded this concept to include systematic review and meta-regression in the establishment of guidelines for cardiovascular risk due to arsenic in drinking water.

The objective of this study was to develop a TSD for benzene by utilizing human data from both epidemiological and other investigations of benzene exposure as well as surrogate animal data from laboratory studies as a comparison. This would be utilised to establish guidelines for human exposure to benzene.

2.0 USE OF TOXICANT SENSITIVITY DISTRIBUTIONS (TSD) TO ESTABLISH GUIDELINE VALUES FOR HUMAN EXPOSURE

Sets of data are available for the toxic effects of exposure to toxicants, including benzene, resulting from laboratory experiments on surrogate animals as well as epidemiological investigations of exposed populations and workers in an occupational setting. In conducting human health risk assessments and setting guideline values there is a need for well conducted and evaluated dose – response relationships with humans. However, for a variety of reasons there are no such relationships available or likely to become available in the near future. On the other hand, the available data for many toxicants on surrogate animals is extensive and reports many useful results but the surrogate animals, usually rats and mice, differ in many ways from a human being. The most obvious differences are in size and life expectancy but there are many other physiological and biochemical differences as well. Thus, the value of this data in setting guideline values for exposure is reduced and usually safety or application factors are applied to compensate for these deficiencies. Use of these factors is necessary but renders the validity of the data in its final adjusted form doubtful although it still can perform a useful function.

On the other hand, the available human data takes many forms but the results of epidemiological investigations are most readily available for chemicals already in use. This data is available from many different investigations which have evaluated different human populations, for different periods of time with different exposure patterns and also evaluate different health end points utilising different study designs. Thus, the toxicological values are derived using no consistent strategy of investigation which would incorporate a consistent pattern of exposure as well as consistent methods to evaluate adverse health effects.

This diversity presents a problem in systematic interpretation to arrive at valid result (Phung et al, 2017). However, the epidemiological data sometimes includes a LOAEL value representing the lowest level for which an adverse effect has been reported. For example, these LOAEL values for benzene from epidemiological investigations constitute a considerable volume of data. If the LOAEL data has not been reported for an investigation the lowest value reported for the investigation can be used. While these values are not the actual experimental value, they can be considered an approximation of this value. This approach has been used by Hamidin et al (2008) and Phung et al (2012).

This lowest observed level for adverse effects from human epidemiological investigations, as well as surrogate animal experiments in the laboratory, can be collated and organised as a sequence with increasing exposure representing a related sequence of adverse health effects. It can be presented as a cumulative probability distribution which is best described as a Toxicant Sensitivity Distribution (TSD). Such TSDs have been prepared by Hamidin et al (2008), Phung et al (2012) and Phung et al (2015).

Such distributions lack the precision of a controlled dose – response relationship but have utility for risk assessment and the setting of guidelines. The TSD can be tested using linear regression analysis between approximately 5 and 95% on a linear – logarithm basis, and the correlation coefficient used as a measure of internal consistency.

However, the TSD plots could be expected to show deviations from this normal distribution since the data are from diverse sources. If the deviations shown in the probabilistic plot are too great, the data in the set could be considered as unsuitable for use. But, if the derived probabilistic plots from the various investigations are linear and suggest a consistent normal distribution, they could be used as a TSD (Phung, 2015).

In deriving guideline values using the LOAEL data as a TSD plot, quantifying the threshold below which adverse effects are not observed and above which there are adverse effects is the objective. Hence the lowest levels of the individual investigations (the LOAELs) are used to derive a TSD. From the linear regression relationship and the trend line alternative methods to establish the threshold are possible which are discussed in Section 4.3. This level is generally accepted as the lowest level that can be consistently estimated from the TSD. It can serve as a guideline value since this threshold is a level above which adverse effects have been reported and may be expected to occur again and below which adverse have not been reported and would not be normally expected. Importantly, in contrast to the safety factor method, the observed exposure in an environmental setting under evaluation can be referred back to the TSD plot which can give an indication of possible adverse health effects previously observed at that level of exposure.

3.0 METHODOLOGY

3.1 Collection of Data

Data was collected from epidemiological studies on human exposure to benzene and data from animal experiment with benzene was also collected. The data sets were compiled from the scientific literature showing measured monitoring data in various studies identified by search databases such as Web of Knowledge, Science Direct, Medline, Pubmed and Google. Criteria for inclusion of a data set were that the investigation was on adults, included data on benzene levels as well as corresponding data on adverse effects as well as identifying the Lowest Observed Adverse Effects Levels (LOAEL). In this current investigation, the benzene concentration is expressed as $\mu\text{g}/\text{m}^3$. A conversion factor of $1\text{ppm} = 3,190\mu\text{g}/\text{m}^3$ was used when converting units from ppm to $\mu\text{g}/\text{m}^3$. Conversions for chemicals in air are made assuming a pressure of 1 atmosphere and a temperature of 25 degrees Celsius (USEPA, 2012b). The data sets were categorised into three groups based on exposure time periods: short term exposure (STE), medium term exposure (MTE) and long-term exposure (LTE) (Table 1 to 5). In addition, the short term human and animal data was categorised as lethal and non-lethal, the medium data as lethal and non-lethal, the long term data as carcinogenic and non-carcinogenic.

3.3 Definition of Short-Term (STE), Medium – Term(MTE) and Long-Term Exposure(LTE)

As defined by ATDSR (2018), acute exposure occurs between 1 to 14 days, intermediate exposures as > 14 to 365 day and chronic exposures as 365 days or more. USEPA (2009) defines acute exposure as <24hrs, sub chronic as > 30 days to 10% of human lifespan and chronic exposure > 10% of human lifespan. Klaassen (2001) reports that, usually the exposure to chemicals is divided into four categories: acute, subacute, sub - chronic, and chronic. Acute exposure is defined as exposure to a chemical for less than 24 hours. Subacute exposure refers to repeated exposure to a chemical for 1 month or less, sub - chronic for 1 to 3 months and chronic for more than 3 months. WHO (2010) defines single and short-term exposures as minutes, hours or a day. Medium-term or intermediate exposure ranges

from weeks to months. Long-term exposures are for which average exposure is for a lifetime. With human and experimental animal studies, WHO (1991) categories short-term exposure as 1 day to 28days, long-term exposure as > 6 months and lifetime as > 1 year.

In this work, the data sets obtained for human and experimental animals were presented in three categories which are short – term exposure {STE, 1–28 days (<1hr/day)}, medium – term exposure {MTE 1– 6 months (8hrs/day)} and long – term exposure {LTE > 6 months (8hrs/day) to lifetime (70years)}. However, the LTE was calculated from the LOAEL as the LOAEL- ADD using Equation 1, thus giving the average daily dose (ADD) for the period of exposure at 8 hrs/day and the LOAEL- LADD using Equation 2, thus giving the ADD for a lifetime. The categories used are similar to the categorisation used in WHO (1991) for short-term exposure as 1 day to 28days, long-term exposure as > 6 months and lifetime as > 1 year.

3.4 Estimation of Exposure Doses

The data obtained from the epidemiological studies on human and experimental animals were reported as concentrations of benzene through inhalation. These data sets were converted to doses in terms of Average Daily Dose (ADD) and Lifetime Average Daily Dose (LADD) by taking into account the inhalation rate and body weight. the Average Daily Dose (ADD) ($\mu\text{g/kg/day}$) was derived using Equation 1, below

$$ADD (\mu\text{g/kg/day}) = [C \times IR \times EF] / [BW] \quad \text{Equation 1}$$

The Lifetime Average Daily Dose (LADD) ($\mu\text{g/kg/day}$) was estimated using the following Equation.

$$LADD (\mu\text{g/kg/day}) = [ADD \times ED] / [LT] \quad \text{Equation 2}$$

where ADD and LADD are dose ($\mu\text{g/kg/day}$); C, concentration in air ($\mu\text{g/m}^3$); IR, inhalation rate (m^3/day); BW, body weight (kg); ED, exposure duration, the period of time over which the person was in contact with the chemical in years but was converted to days (d) for this study; EF, exposure frequency, the frequency of exposure in days/years but was converted to days/day (d/d); LT, lifetime average exposure (70years) converted to days (70 x 365).

3.5 Estimation of Human Equivalent Dose (HED)

The data for evaluating dose-response relationships are usually obtained from laboratory experiments on surrogate animal. This toxicity data, obtained from the scientific literature, was converted to Human Equivalent Dose (HED) based on Body Surface Area (BSA) and Body Weight (BW) using Equation 3 recommended by the US Food and Drug Administration (USFDA). This was carried out by using a correction factor that reflects the relationship between body weight and body surface area (Human K_m and Animal K_m). K_m factor (kg/m^2) (USFDA, 2005).

$$HEADD \text{ or } HELADD (\mu\text{g/kg/day}) = [AD] \times [K_{m_{\text{Animal}}} / K_{m_{\text{Human}}}] \quad \text{Equation 3}$$

where, HEADD is Human Equivalent Average Daily Dose ($\mu\text{g/kg/day}$); HELADD is Human Equivalent Lifetime Average Daily Dose ($\mu\text{g/kg/day}$); AD, animal dose ($\mu\text{g/kg/day}$); $K_{m_{\text{Human}}}$ and $K_{m_{\text{Animal}}}$, K_m factors for human and animals (kg/m^2).

3.6 Developing Guidelines for Benzene Using Toxicant Sensitivity Distributions (TSD)

The data on benzene available from various epidemiological studies for humans and animal experiments (Tables 1 to 5) were used to develop TSD plots using Equation 4, below

$$CP(\%) = i/[n + 1] \times 100 \quad \text{Equation 4}$$

where, CP is cumulative probability (%); i, ith point; n, total number of points.

From the TSD plots, the linear regression equations were calculated between approximately 5 – 95% of the CPLED since this represents the approximately linear part of the CPLED plots, where 5%CP can be estimated. The 5%CP level is preferred as the lowest level that can be reliably estimated from data using the CPLED relationships (Phung et al. 2015).

The 5%CP values obtained from this relationship for STE and LTE were in $\mu\text{g/kg/day}$. Values in $\mu\text{g/kg/day}$ were converted to $\mu\text{g/m}^3$ using Equation 5 as shown below.

$$C (\mu\text{g/m}^3) = [ADD \times BW]/[IR] \quad \text{Equation 5}$$

4.0 RESULTS AND DISCUSSION

4.1 Health Effects of Exposure to Benzene

The adverse effects from exposure to benzene, such as haematotoxicity, immunotoxicity, genotoxicity and carcinogenicity both in humans and animals, are summarized in Tables 1 to 5 which also includes effects such as pancytopenia, lymphocytopaenia, anaemia, leukopenia, aplastic anaemia and thrombocytopenia (Cody et al. 1993; Lan et al. 2004a; Qu et al. 2002; Kipen et al. 1989; Xia et al. 1995). Death as a result short – term exposure has been reported in the epidemiological studies of (Flurry 1928; Greenberg 1926; Midzenski et al. 1992; Alvis and Hutton 1993). Long – term exposure to benzene causes damage to the bone marrow that can result in a decrease in the numbers of circulating blood cells, and aplastic anaemia (Baak 1999; Yin et al. 1987c; Snyder et al. 1980). However other studies on human and animals have indicated the development of different types of leukaemia (Cronkite et al. 1985; Maltoni et al. 1982a; Maltoni et al. 1985; Yin et al. 1989).

The cohort Pliofilm study of industrial workers (Paxton et al. 1994a; Paustenbach et al. 1992) and Chinese cohort study of workers (Yin et al. 1996) occupationally exposed to benzene suggest a causal relationship between exposure to benzene and the development of leukaemia. Aksoy (1987) and Snyder et al. (1984) have suggested that the principal carcinogenic response to long – term human exposure to benzene is leukaemia while animal experiment studies have indicated solid tumours in specific organs of the animal species. On the other hand, no clear associations were found at either low or high doses of benzene and the risk of lymphoma (non-Hodgkin's Lymphoma and Multiple (Smith et al 2007; Kane and Newton, 2010).

4.2 Evaluation of Exposure Dose using the ADD and LADD

The available exposure data was in the form of LOAEL values which required conversion to doses to allow the inclusion of information on body weight, exposure duration and other factors (see Section 3.4). Exposure doses can be evaluated in several different ways however the most common measures of dose for risk assessment are Average Daily Dose (ADD) (Equation 1) and Lifetime Average Daily Dose (LADD) (Equation 2). ADD is normally used for calculating non-cancer risks over periods up to the actual exposure duration. On the other hand, the LADD is usually used to evaluate carcinogenic effects over the long term generally a lifetime (70 years). As shown in ATSDR (2005) and WHO (2010), the difference between these two measures is the time period over which the calculation is made. In this work, the ADD was used in estimating the non-carcinogenic effects of benzene with the average exposure during the period of contact with benzene as the actual exposure duration. In situations where the dominant health risk for exposure to benzene is cancer, the exposure dose (LADD) is often described in terms of lifetime average exposure (70 years). In most situations, the exposure to benzene does not occur continuously for a lifetime, however, it is assumed that carcinogenic effects are cumulative over this period. In the utilization of animal data, the toxicity data were converted to Human Equivalent Dose (HED) (see Section 3.5).

4.3 TSD Relationships of LOAEL with Dose of Benzene

TSD Relationships from Human Epidemiological Data

The adverse effects for short –term exposure (STE) and long – term exposure (LTE) to benzene from human epidemiological data are summarized in Table 1 and 2. The data in Table 1 is insufficient to enable any meaningful plots to be made but it is indicative of possible adverse effects. The data in Table 2 is plotted as a TSD plot of the LOAEL-LADD (Figure 1). Linear regression relationship from 5 to 95%CP gave a high correlation coefficient values, R^2 0.95. The chronic inhalation reference dose for USEPA (8.5µg/kg/day) was above several of the lowest LOAEL – ADD values (Figure 1). This suggests that adverse health have occurred in human populations at exposure levels below this reference dose.

TSD Relationships from Animal Experiment Toxicity Data

Animal toxicity data for short –term exposure (STE), medium – term exposure (MTE) and long – term exposure (LTE) to benzene are summarized in Table 3, 4 and 5 and the data was plotted as TSD plots of LOAEL-HEADD (Figure 2 and Figure 3). Correlation coefficient (R^2) values for the 5 to 95% range were from 0.69 to 0.96 as shown in Figures 2 and 3. With this animal data, the USEPA inhalation reference dose RfD (8.5µg/kg/day) for chronic exposure to benzene was much lower than the minimum LOAEL – HEADD values of 3600µg/kg/day and 11000µg/kg/day.

4.4 Estimating 5%CP in a TSD Relationship

This method utilises the linear section of the trend line, which is reliably characterised by a linear regression equation between approximately 5% - 95%CP of the TSD plot, at the 5%CP level. The TSD plots all follow the

normal distribution pattern since the R^2 values are high at 0.95 (32 points, Table 2, Figure 1) and 0.97 (32 points, Table 2, Figure 4C)

4.5. Estimation of the Safety Ratio of Animal to Human from TSD Relationships

The information from human epidemiology studies provides relevant and reliable toxicity for the risk assessment process. However human data are not commonly available and animal toxicity data are usually utilized. This requires the use of a safety factor (SF) or uncertainty factor (UF) is required to make the animal toxicity data usable for situations faced by humans. However, the use of SF or UF also has some disadvantages. Firstly, there is no data available to allow quantification of the magnitude required apart from a set of qualitative rules. Also, it is derived from surrogate animal data which may not be applicable. In addition, the nature of the adverse effects which can be expected by cannot be defined.

Surrogate animal data is principally used in setting guideline values for exposure to benzene. These are often derived by applying a Safety Factor to the data to account for factors such as data from animals to humans, different species, different experiments, different exposure times, different endpoints and the suitability of existing data. There has not been any quantitative evaluation of the validity of these Safety Factors. This represents the first occasion when such an evaluation has been possible. Thus, the Safety Ratio (SR) was calculated using human and animal data to compare with the actual safety factors used with setting guideline for benzene. The Safety Ratio values were estimated at 5% and 50%CP as the ratio of the animal data to the human data (LOAEL-HEADD/LOAEL-ADD) in terms of ADD and the ratio of animal data to human data (LOAEL-HELADD/LOAEL-LADD) in terms of LADD from Figure 4.

The SR for long – term exposure to benzene (ADD, 11 and 100; 0.25 and 2.5) was compared to the Uncertainty Factor for deriving USEPA Reference Dose (RfD). An uncertainty factor of 300 was applied in estimating the Reference Concentration (RfC). This was approximately 3 to 1,200 times higher than the value of the Safety Ratio derived in this study for LTE in terms of ADD (3.5 and 100) and LADD (0.25 and 2.5). This suggests that the Uncertainty Factor used may have been greater than necessary.

4.6 Proposed Guideline Values Relating to Exposure Dose

It is considered that the actual human data is more appropriate to use in setting guidelines since this data is directly relevant to setting of guidelines rather than use of the animal data with a safety factor. It is noteworthy that the CP is not related to the actual human population exhibiting adverse effects but it is the CP of the different studies used in developing the TSD plots. However, the TSD plots place the toxicity data in a sequence of increasing toxic effects which can be used for predictive purposes. Hence, GVs can be derived for LTE using the LOAEL–ADD at 5%CP of the TSD relationship of Figure 4C. The 5%CP represents the minimum of the reliable LOAEL values in the CPLED plot and can be considered as a guideline value (Phung et al., 2015). Below the guideline, there are no reliable records of adverse effects and above this value, adverse effects have been reliably detected. The value obtained at 5%CP for STE was approximately 510 μ g/kg/day. However, this value was derived from only 5 data points. This

number of points is regarded insufficient for a reliable result. The guideline value obtained at 5%CP for LTE in terms of ADD was 92µg/kg/day and 3.4µg/kg/day (Figure 1) for lifetime average exposure (70years) in terms of LADD.

For comparison purposes, the derived Guideline Value at 5%CP for LTE in terms of ADD (92µg/kg/day) and LADD (3.4µg/kg/day) were converted from dose values in µg/kg/day to concentration values in µg/m³ using Equation 5 as most of the Guideline Values are in terms of concentration in air. The derived Guideline Values were compared with sets of Guideline Values for both occupational exposure and general public exposure. The derived guideline value at 5%CP for LTE in terms of ADD was compared with occupational exposure limits (OEL) based on the assumption that occupational exposure is on a daily basis over a specific period of time. LTE in terms of ADD relates to daily exposure to benzene in occupational environment. The suggested occupational exposure limit for LTE (8hr/day) exposure to benzene is 320µg/m³ and 280µg/m³ for LTE (10hr/day) exposure. Lifetime average exposure in terms of LADD (12µg/m³) was compared with air quality guideline (AQG) for chronic exposure. This guideline value in terms LADD relates to chronic exposure for general population based on the assumption that exposure for general population could be for a lifetime. Hence this derived value (12µg/m³) is suggested as an air quality guideline value for general population exposure to benzene.

5.0 CONCLUSIONS

For humans, leukaemia was the most common type of cancer reported for exposure to benzene while other types of cancers were reported from animal studies. The concept of the 5%CP provides an alternative method for estimating guideline values using human data as a Toxicant Sensitivity Distribution (TSD). It differs from current methods used to derive guideline values by regulatory organisations which use Safety Factors and different biological endpoints. TSD were the linear regression equations obtained from plots of the LOAEL at long term (LTE) and lifetime exposure which were calculated from the same data set (Table 2) as the LOAEL-ADD and LOAEL-LADD. These TSD for LOAEL at long term (LTE) and lifetime exposure were based on sufficient data points (32) and gave the following linear regression equations

$$\text{CP (\%)} = 28 \log (\text{LOAEL} - \text{ADD}) - 50 \quad (\text{Figure 4C}) \quad R^2 = 0.97$$

$$\text{CP (\%)} = 25 \log (\text{LOAEL-LADD}) - 8.1 \quad (\text{Figure 1}) \quad R^2 = 0.96$$

The exposure was estimated at 5%CP which has been suggested as a guideline level. Guideline Values obtained were 92µg/kg/day for long term exposure (LTE) at 8hr/day which is appropriate for occupational exposure and 3.4µg/kg/day for lifetime exposure (70yrs) for general population exposure to benzene. The guideline of 3.4µg/kg/day using the LADD is a new guideline not used by regulatory agencies but indicated by this analysis with the available data from human epidemiological studies. This guideline value when converted to concentration in air (12µg/m³) can be compared to the European Union (5µg/m³), ATSDR (10µg/m³) and EPAQS (16µg/m³), USEPA (30µg/m³), OEHHA (60µg/m³) air quality guideline for general population by the regulatory agencies. Safety ratio values describing the relationships of human epidemiological data and animal toxicity data ranging from 0.25 to 100

was estimated from the CPLED plots are somewhat lower than that used by regulatory agencies usually from 10 to 1000 suggesting more information is required when applying safety factors.

Acknowledgments

This work was supported by Griffith University Australia Postgraduate Scholarship Award (scholarship for doctoral study) and Delta State Government Nigeria.

6.0 REFERENCE

- ATSDR (2018) Minimal Risk Levels (MRLs) – For Professionals.
<https://www.atsdr.cdc.gov/mrls/index.asp> (accessed 09.09.18)
- 2018.Aksoy M (1987) Chronic lymphoid leukaemia and hairy cell leukaemia due to chronic exposure to benzene: Report of three cases. *Br J Haematol.* 66: 209-211
- Aksoy M, Erdem S (1978) Followup study on the mortality and the development of leukemia in 44 pancytopenic patients with chronic benzene exposure. *Blood* 52: 285-292
- Aksoy M, Dincol K, Erdem S, (1972) Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. *Br J Ind Med* 29: 56-64
- Avis SP, Hutton CJ (1993) Acute benzene poisoning: A report of three fatalities. *J Forensic Sci* 38(3): 599-602
- Aoyama K (1986) Effects of benzene inhalation on lymphocyte subpopulations and immune response in mice. *Toxicol Appl Pharmacol* 85: 92-101
- Baak YM, Ahn BY, Chang HS, Kim JH, Kim KA, Lim Y (1999) Aplastic anemia in a petrochemical factory worker. *Environ Health Perspect* 107(10):851-3
- Baarson KA, Snyder CA, Albert RE (1984) Repeated exposures of C57B1 mice to inhaled benzene at 10 ppm markedly depressed erythropoietic colony formation. *Toxicol Lett* 2: 337-342
- Bogadi-Šare A, Brumen V, Turk R (1997) Genotoxic effects in workers exposed to benzene: With special reference to exposure biomarkers and confounding factors. *Ind Health* 35: 367-373
- Carpenter CP, Shaffer CB, Weil CS (1944) Studies on the inhalation of 1:3-butadiene; with a comparison of its narcotic effect with benzol, toluol, and styrene, and a note on the elimination of styrene by the human. *J Ind Hyg Toxicol* 26: 69-78
- Cao Q, Yu Q, Des W (2011) Health risk characterisation for environmental pollutants with a new concept of overall risk probability. *Journal of Hazardous Materials* 187: 480–487
- Chen D, Cho SI, Chen C, Wang X, Damokosh A, Ryan L, Smith TJ, Christiani DC, Xu X (2000). Exposure to benzene, occupational stress and reduced birth weight. *Occup Environ Med* 57: 661- 667
- Chertkov JL, Lutton JD, Jiang S (1992) Hematopoietic effects of benzene inhalation assessed by murine long-term bone marrow culture. *J Lab Clin Med* 119: 412-419.
- Coate WB, Hoberman AM, Durloo RS (1984) Inhalation teratology study of benzene in rats. *Adv Mod Environ Toxicol* 6: 187-198
- Cody RP, Strawderman WW, Kipen HM (1993) Hematologic effects of benzene. Job-specific trends during the first year of employment among a cohort of benzene-exposed rubber workers. *J Occup Med* 35(8): 776-782

- 365 Collins JJ, Ireland B, Buckley CF (2003) Lymphohaematopoietic cancer mortality among workers with benzene
366 exposure. *Occup Environ Med* 60(9): 676-679
- 367 Cronkite EP (1986) Benzene hematotoxicity and leukemogenesis. *Blood Cells* 12: 129-137
- 368 Cronkite EP, Drew RT, Inoue T (1985) Benzene hematotoxicity and leukemogenesis. *American Journal of Industrial*
369 *Medicine* 7: 447-456
- 370 Cronkite EP, Drew RT, Inoue T (1989). Hematotoxicity and carcinogenicity of inhaled benzene. *Environ Health*
371 *Perspect* 82: 97-108
- 372 Deichmann WB, MacDonald WE, Bernai E (1963) The hemopoietic tissue toxicity of benzene vapors. *Toxicol.*
373 *Appl. Pharmacol.*, 5: 201-224
- 374 Dempster AM, Snyder CA (1991) Kinetics of granulocytic and erythroid progenitor cells are affected differently by
375 short-term, low-level benzene exposure. *Arch Toxicol* 65(7): 556-561
- 377 Djohan D, Yu J, Connell D, Christensen E (2007) Health risk assessment of chlorobenzenes in the air of residential
378 houses using probabilistic techniques. *J. Toxicol. Environ. Health* 70: 1594-1603
- 379 Doskin VA (1971) Effect of age on the reaction to a combination of hydrocarbons. *Hyg Sanit* 36: 379-384
- 380 Dow (1992) Effects of benzene vapor in the pig and rat. I. Pertaining to hematology and immunology with cover
381 letter dated 05/14/92. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E.
382 OTS0539784
- 383 Drew RT, Fouts JR (1974) The lack of effects of pretreatment with phenobarbital and chlorpromazine on the acute
384 toxicity of benzene in rats. *Toxicol Appl Pharmacol* 27: 183-193
- 385 Edokpolo B, Qiming JY, Connell D (2014) Health Risk Assessment of Ambient Air Concentrations of Benzene,
386 Toluene and Xylene (BTX) in Service Station Environments. *Int. J. Environ. Res. Public Health* 11(6): 6354-6374
- 387 Edokpolo B, Yu QJ, Connell D (2015) Health risk assessment for exposure to benzene in petroleum refinery
388 environments. *Int J Environ Res Public Health*. 12(1): 595-610
- 390 enHEALTH (2012) Environmental Health Risk Assessment. Guidelines for assessing human health risks from
391 environmental hazards. Department of Health and Ageing and enHealth Council, ACT, Australia.
- 393 Farris GM, Everitt JI, Irons RD (1993) Carcinogenicity of inhaled benzene in CBA mice. *Fundam Appl Toxicol*
394 20(4): 503-507
- 395 Farris GM, Robinson SN, Gaido KW (1997) Benzene-induced hematotoxicity and bone marrow compensation in
396 B6C3F1 mice. *Fundam Appl Toxicol* 36(2): 119-129
- 397 Fishbeck WA, Townsend JC, Swank MG (1978) Effect of chronic occupational exposure to measured
398 concentrations of benzene. *J Occup Med* 20: 539-542
- 399 Flury F (1928) Toxicities in modern industry. Ila. Pharmacological-toxicological aspects of intoxicants in modern
400 industry. *Arch Exp Pathol Pharmacol* 138: 65-82
- 401 Gill DP, Jenkins VK, Kempen RR (1980) The importance of pluripotential stem cells in benzene toxicity.
402 *Toxicology* 16: 163-171
- 403 Goldwater LJ (1941) Disturbances in the blood following exposure to benzol. *J Lab Clin Med* 26: 957 – 973
- 404 Green JD, Leong BKJ, Laskin S (1978) Inhaled benzene fetotoxicity in rats. *Toxicol Appl Pharmacol* 46: 9-18

- 405 Green JD, Snyder CA, LoBue J (1981a) Acute and chronic dose/response effects of inhaled benzene on
 406 multipotential hematopoietic stem (CFU-S) and granulocyte/macrophage progenitor (GMCFU-C) cells in CD-1
 407 mice. *Toxicol Appl Pharmacol.* 58: 492-503
- 408 Greenburg L, Mayers MR, Goldwater L (1939) Benzene (benzol) poisoning in the rotogravure printing industry in
 409 New York City. *J Ind Hyg Toxicol.* 21: 395-420
- 410 Hamidin N, Yu QJ, Connell DW (2008) Human health risk assessment of chlorinated disinfection by-products in
 411 drinking water using a probabilistic approach, *Water Res.* 42: 3263-3274
- 412
 413 IARC (2010) International Agency for Research on Cancer (IARC)—Summaries & evaluations. Lyon, International
 414 Agency for Research on Cancer (<http://www.inchem.org/pages/iarc.html>, accessed 20 July 2014).
- 415
 416 IARC (2012) International Agency for Research on Cancer (IARC), International Programme on Chemical Safety
 417 Benzene – IARC Monograph, pp 249 – 285.
- 418
 419 Infante PF, Rinsky RA, Wagoner JK (1977) Leukemia in benzene workers. *Lancet.* 2: 76-78
- 420 Infante PF (1978) Leukemia among workers exposed to benzene. *Tex Rep Biol Med.* 37: 153-161
- 421 Kane EV, Newton R (2010) Occupational exposure to gasoline and the risk of non-Hodgkin lymphoma: A review
 422 and meta-analysis of the literature. *Cancer Epidemiology* (34)5: 516-522
- 423 Keller KA, Snyder CA (1988) Mice exposed in utero to 20 ppm benzene exhibit altered numbers of recognizable
 424 hematopoietic cells up to seven weeks after exposure. *Fundam Appl Toxicol.* 10: 224-232
- 425 Kipen HM, Cody RP, Goldstein BD (1989) Use of longitudinal analysis of peripheral blood counts to validate
 426 historical reconstructions of benzene exposure. *Environ Health Perspect.* 82: 199-206
- 427 Klaassen DC (2001) Casarett and Doull's Toxicology. The Basic Science of Poisons Seventh Edition. McGraw-Hill
 428 Medical Publishing Division New York Chicago San Francisco Lisbon London Madrid Mexico City New Delhi San
 429 Juan Seoul Singapore Sydney Toronto
- 430 Kuna RA, Kapp RW (1981) Embryotoxic/teratogenic potential of benzene vapor in rats. *Toxicol Appl Pharmacol.*
 431 57: 1-7
- 432 Lan Q, Zhang L, Li G, Vermeulen R, Weinberg RS, Dosemeci M, Rappaport SM, Shen M, Alter BP, Wu Y, Kopp
 433 W, Waidyanatha S, Rabkin C, Guo W, Chanock S, Hayes RB, Linet M, Kim S, Yin S, Rothman N, Smith MT
 434 (2004a) Hematotoxicity In Workers Exposed To Low Levels Of Benzene. *Science* 306 (5702): 1774-1776
- 435
 436 Li GL, Yin SN, Watanabe T (1986) Benzene-specific increase in leukocyte alkaline phosphatase activity in rats
 437 exposed to vapors of various organic solvents. *J Toxicol Environ Health* 19: 581-589
- 438 Luke CA, Tice RR, Drew RT (1988b) The effect of exposure regimen and duration on benzene-induced bone
 439 marrow damage in mice: II. Strain comparisons involving B6C3F1, C57BL/6 and DBA/2 male mice. *Mutat Res.*
 440 203: 273-295
- 441 Maltoni C, Cotti G, Valgimigli L (1982a.) Hepatocarcinomas in Sprague-Dawley rats following exposure to benzene
 442 by inhalation: First experimental demonstration. *Med Lav.* 4: 446-450
- 443 Maltoni C, Conti B, Cotti G (1985) Experimental studies on benzene carcinogenicity at the Bologna Institute of
 444 Oncology: Current results and ongoing research. *Am J Ind Med.* 7: 415-446

- 445 Midzenski MA, McDiarmid MA, Rothman N (1992) Acute high dose exposure to benzene in shipyard workers. *Am*
446 *J Ind Med.* 22: 553-565
- 447 Neun DJ, Penn A, Snyder CA (1992) Evidence for strain-specific differences in benzene toxicity as a function of
448 host target cell susceptibility. *Arch Toxicol.* 66(1): 11-17
- 449 Ott MG, Townsend JC, Fishbeck WA (1978) Mortality among workers occupationally exposed to benzene. *Arch*
450 *Environ Health.* 33: 3-10
- 451 Paustenbach DJ, Price PS, Ollison W (1992) Reevaluation of benzene exposure for the pliofilm(rubberworker)
452 cohort (1936-1976). *J Toxicol Environ Health.* 36(3): 177-231
- 453 Paxton MB, Chinchilli VM, Brett SM (1994a) Leukemia risk associated with benzene exposure in the pliofilm
454 cohort: I. mortality update and exposure distribution. *Risk Analysis* 14(2): 147-154
- 455 Phung DT, Connell D, Miller G, Chu C (2012) Probabilistic assessment of chlorpyrifos exposure to rice farmers in
456 Vietnam. *Journal of Exposure Science and Environmental Epidemiology* 22: 417-423
- 457 Phung DT, Connell D, Chu C (2015) New method to set guidelines to protect human health from agricultural
458 exposure using chlorpyrifos as an example. *Annals of Agricultural and Environmental Medicine* 22 (2): 274-279
459
- 460 Phung DT, Connell D, Chu C (2016). Correspondence to the Editor Re: Implementing systematic review
461 techniques in chemical risk assessment: Challenges, opportunities and recommendations. *Environment International*
462
- 463 Phung DT, Connell D, Rutherford S, Chu C (2017). Cardiovascular risk from water arsenic exposure in Vietnam -
464 Application of systematic review and meta-regression analysis in chemical health risk assessment *Chemosphere*
465 177, 167 – 175.
466
- 467 Plappert U, Barthel E, Raddatz K (1994a) Early effects of benzene exposure in mice. *Haematological versus*
468 *genotoxic effects.* *Arch Toxicol.* 68: 284-290
- 469 Qu Q, Shore R, Li G (2002) Hematological changes among Chinese workers with a broad range of benzene
470 exposures. *Am J Ind Med.* 42(4): 275-285
- 471 Raw GJ, Coward SKD, Brown VM, Crump DR (2004) Exposure to air pollutants in English homes. *J Expo. Anal.*
472 *Environ. Epidemiol.* 14: 85-94
473
- 474 Rinsky RA, Young RJ, Smith AB (1981) Leukemia in benzene workers. *Am J Ind Med.* 2: 217-245
- 475 Robinson SN, Shah R, Wong BA (1997) Immunotoxicological effects of benzene inhalation in male Sprague-
476 Dawley rats. *Toxicology* 119(3): 227-237
- 477 Rosenthal GJ, Snyder CA (1987) Inhaled benzene reduces aspects of cell-mediated tumor surveillance in mice.
478 *Toxicol Appl Pharmacol* 88: 35-43
- 479 Rothman N, Li GL, Dosemeci M (1996a) Hematotoxicity among Chinese workers heavily exposed to benzene. *Am J*
480 *Med* 29(3): 236-246
- 481 Singer BC, Hodgson AT, Nazaroff WW (2003) Gas-phase organics in environmental tobacco smoke: 2. Exposure-
482 relevant emission factors and indirect exposures from habitual smoking. *Atmospheric Environment* 37: 5551
483
- 484 Smith MT, Jones RM, Smith AH (2007) Benzene exposure and risk of non-Hodgkin lymphoma. *Cancer Epidemiol*
485 *Biomarkers* 16(3): 385-91
- 486 Smyth HF, Carpenter CP, Weil CS (1962) Range-finding toxicity data: List VI. *Am Ind Hyg Assoc J.* 23: 95-107

- Snyder CA, Goldstein BD, Sellakumar A (1982) Toxicity of chronic benzene inhalation: CD-1 mice exposed to 300 ppm. *Bull Environ Contam Toxicol*. 29: 385-391
- Snyder CA, Goldstein BD, Sellakumar AR (1984) Evidence for hematotoxicity and tumorigenesis in rats exposed to 100 ppm benzene. *Am J Ind Med*. 5: 429-434
- Snyder CA, Goldstein BD, Sellakumar AR (1980) The inhalation toxicology of benzene: Incidence of hematopoietic neoplasms and hematotoxicity in AKR/J and C57BL/6J mice. *Toxicol Appl Pharmacol*. 54: 323-331
- Snyder CA, Sellakumar AR, James DJ (1988) The carcinogenicity of discontinuous inhaled benzene exposures in CD-1 and C57BL/6 mice. *Arch Toxicol*. 62: 331-335
- Snyder CA, Goldstein BD, Sellakumar A (1978a) Hematotoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. *J Toxicol Environ Health* 4: 605-618
- Thurston SW, Ryan L, Christiani DC, Snow R, Carlson J, You L, Cui S, Ma G, Wang L, Huang Y, Xu X (2000). Petrochemical exposures and menstrual disturbances. *Am J Ind Med* 38: 555-564
- USEPA (2002) Toxicological Review of Benzene (Noncancer Effects). U. S. Environmental Protection Agency.
- USEPA (2005) Guidelines for carcinogen risk assessment. EPA/630/P-03/001F. Washington DC, U. S. Environmental Protection Agency.
- USEPA (2009) Risk assessment guidance for superfund Volume 1: Human health evaluation manual (Part F, supplemental guidance for inhalation risk assessment).
- USEPA (2012a). Chemical Specific Reference Values for Benzene (CASRN 71-43-2), EPA/600/R-12/047F1, National Center for Environmental Assessment, Office for Research and Development, U.S. Environmental Protection Agency.
- USEPA (2012b) Integrated Risk Information System (IRIS). Substance file - benzene. Washington, DC (January 1): National Center for Environmental Assessment.
- USFDA (2005) Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, Food and Drug Administration, U.S. Department of Health and Human Services, (accessed 09.06.10) <http://www.fda.gov/cder/guidance/index.htm>.
- Vigliani EC, Forni A (1976) Benzene and leukemia. *Environ Res*. 11: 122-127
- Ward CO, Kuna RA, Snyder NK (1985) Subchronic inhalation toxicity of benzene in rats and mice. *Am J Ind Med*. 7: 457-473
- Weisel CP, Alimokhtari S, Sanders PF (2008) Indoor air VOC concentrations in suburban and rural New Jersey. *Environ. Sci. Technol*. 42: 8231-82
- Wells MS, Nerland DE (1991) Hematotoxicity and concentration-dependent conjugation of phenol in mice following inhalation exposure to benzene. *Toxicol Lett*. 56(1-2): 159-166
- WHO (1991) Benzene. Environmental Health Criteria. World Health Organization, Geneva.

- 531 WHO (2010) WHO human health risk assessment toolkit: Harmonization Project Document No. 8 Chemical
532 Hazards.
533
- 534 WHO (2010) Indoor air quality: selected pollutants. World Health Organization Regional Office for Europe, 2010.
535 Guidelines Copenhagen, Denmark.
536
- 537 Xia ZL, Xi Peng J, Pei-Lian L (1995) Ascertainment corrected prevalence rate (ACPR) of leukopenia in workers
538 exposed to benzene in small-scale industries calculated with capture-recapture methods. *Biomed Environ Sci.* 8: 30-
539 34
- 540 Yardley-Jones A, Anderson D, Jenkinson PC (1988) Genotoxic effects in peripheral blood and urine of workers
541 exposed to low level benzene. *Br J Ind Med.* 45: 694-700
- 542 Yin S-N, Hayes RB, Linet MS, Li G-L, Dosemeci M, Travis LB, Li C-Y, Zhang Z-N, Li D-G, Chow W-H,
543 Wacholder S, Wang Y-Z, Jiang Z-L, Dai T-R, Zhang W-Y, Chao X-J, Ye P-Z, Kou Q-R, Zhang X-C, Lin X-F,
544 Meng J-F, Ding C-Y, Zho J-S, Blot WJ (1996) A cohort study of cancer among benzene-exposed workers in China:
545 Overall results. *American Journal of Industrial Medicine* 29:227-235
- 546 Yin SN, Li GL, Tain FD (1989) A retrospective cohort study of leukemia and other cancers in benzene workers.
547 *Environ Health Perspect.* 82: 207-213
- 548 Yin SN, Li Q, Liu Y (1987c) Occupational exposure to benzene in China. *Br J Ind Med.* 44: 192-195
- 549 Yu QJ, Cao Q, Connell D (2012) An overall risk probability-based method for quantification of synergistic and
550 antagonistic effects in health risk assessment for mixtures: theoretical concepts. *Environmental Science and*
551 *Pollution Research* 19: 2627-2633

Table 1 Reported toxicity data for short – term human exposure to benzene

LETHAL EFFECTS					
Reference	LOAEL ($\mu\text{g}/\text{m}^3$)	ED (d)	EF (d/d)	LOAEL– ADD ($\mu\text{g}/\text{kg}/\text{day}$)	Observed Health Effects
Flurry (1928)	6.38×10^7	1	0.01	1.82×10^5	Death
NON – LETHAL EFFECTS					
Reference	LOAEL ($\mu\text{g}/\text{m}^3$)	ED (d)	EF (d/d)	LOAEL– ADD ($\mu\text{g}/\text{kg}/\text{day}$)	Observed Health Effects
Midzenski et al. (1992)	1.91×10^5	1	0.1	5.47×10^3	Drowsiness, dizziness, headaches, strong odour, fatigue
Flurry (1928)	9.57×10^5	0.5	0.04	1.09×10^4	Drowsiness, headaches, dizziness
Midzenski et al. (1992)	191×10^5	1	0.1	5.47×10^3	Mucous membrane irritation
Midzenski et al. (1992)	1.91×10^5	21	0.33	1.81×10^4	Mucous membrane irritation, dyspnea

LOAEL – Lowest Observed Adverse Effects Levels

ED – Exposure Duration (day)

EF – Exposure length (day/day)

LOAEL–ADD - Lowest Observed Adverse Effects Average Daily Dose

Table 2. Reported toxicity data for long – term human exposure to benzene

Reference	LOAEL ($\mu\text{g}/\text{m}^3$)	ED (d)	EF (d/d)	LOAEL–ADD ($\mu\text{g}/\text{kg}/\text{day}$)	LOAEL–LADD ($\mu\text{g}/\text{kg}/\text{day}$)	Observed Health Effect
Aksoy and Erdem, (1978)	4.79×10^5	5475	0.33*	4.51×10^4	96.68×10^3	Hematotoxicity – Pancytopenia
Doskin, (1971)	9.57×10^3	1095	0.33*	9.02×10^2	3.87×10^1	Hematotoxicity – Anemia, lymphocytosis, leukopenia,
Aksoy et al., (1972)	6.69×10^5	365	0.33*	6.32×10^4	9.02×10^2	Hematotoxicity – Pancytopenia, hypercellular bone marrow
Cody et al., (1993)	1.28×10^5	365	0.33*	1.21×10^4	1.71×10^2	Hematotoxicity – Decrease in white blood cells counts
Yin et al., (1987c)	9.25×10^4	6935	0.33*	8.72×10^3	2.38×10^3	Hematotoxicity – Aplastic, anemia
Baak, (1999)	6.38×10^1	7665	0.33*	6.02	1.80	Hematotoxicity – Aplastic, anemia
Fishbeck et al., (1978)	7.98×10^4	10585	0.33*	7.52×10^3	3.12×10^3	Hematotoxicity – Increased mean corpuscular volume
Lan et al., (2004a)	1.82×10^3	2190	0.33*	1.71×10^2	1.46×10^1	Hematotoxicity – Reduced WBC and platelet counts
Qu et al., (2002)	7.21×10^3	3541	0.33*	6.79×10^2	9.42	Hematotoxicity – Reduced neutrophils and RBC counts
Rothman et al., (1996a)	2.42×10^4	2300	0.33*	2.29×10^3	2.06×10^2	Hematotoxicity – Reduced absolute lymphocyte count
Aksoy et al., (1972)	6.69×10^5	365	0.33*	6.32×10^4	9.02×10^2	Immunological – hypoplastic to hyperplastic bone marrow
Cody et al., (1993)	1.28×10^5	365	0.33*	1.21×10^4	1.71×10^2	Immunological – Decreased lymphocytes
Goldwater, (1941)	3.53×10^4	1852	0.33*	3.33×10^3	2.41×10^2	Immunological – Anemia, macrocytosis, thrombocytopenia
Kipen et al., (1989)	2.39×10^5	9125	0.33*	2.26×10^4	8.06×10^3	Immunological – Leukopenia
Aksoy et al., (1972)	6.67×10^5	365	0.33*	6.29×10^4	8.99×10^2	Immunological – Pancytopenia
Cody et al., (1993)	1.28×10^5	365	0.33*	1.21×10^4	1.71×10^2	Immunological – Decreased lymphocytes
Greenburg et al., (1939)	3.51×10^4	1852	0.33*	3.31×10^3	2.39×10^2	Immunological – Anemia, macrocytosis, thrombocytopenia
Xia et al., (1995)	2.20×10^3	365	0.33*	2.08×10^2	2.97	Immunological – Leukopenia
Bogadi-Sare et al., (1997)	6.06×10^3	6205	0.33*	5.71×10^2	1.38×10^2	Reproductive – increased in chromosome aberration
Yardley – Jones, (1990)	3.19×10^3	2190	0.33*	3.01×10^2	2.58×10^1	Reproductive – increased in chromosome aberration
Chen et al., (2000)	6.87×10^1	2210	0.33*	6.48	0.56	Reproductive – increased in chromosome aberration
Aksoy and Erdem, (1978)	4.79×10^5	5475	0.33*	4.51×10^4	9.67×10^3	Cancer – Leukemia
Collins et al., (2003)	3.19×10^5	2590	0.33*	3.01×10^4	3.05×10^3	Cancer – Leukemia
Yin et al.,						Cancer – Human lymphocytic

(1987c)	9.25×10^4	6935	0.33*	8.72×10^3	2.37×10^3	leukemia
Aksoy et al., (1987)	3.19×10^3	14600	0.33*	3.01×10^2	1.72×10^2	Cancer – Leukemia
Ott et al., (1978)	9.57×10^2	548	0.33*	9.02	1.94	Cancer – Leukemia
Rinsky et al., (1981)	5.1×10^4	5110	0.33*	4.81×10^3	9.62×10^2	Cancer – Leukemia
Thurston et al., (2000)	2.74×10^3	2555	0.33*	2.59×10^2	2.58×10^1	Cancer – Acute myeloid leukemia
Infante et al., (1977)	5.1×10^4	5110	0.33*	4.81×10^3	9.62×10^2	Cancer – Leukemia
Yin et al., ((1989)	6.87×10^3	365	0.33*	6.48×10^2	9.25	Cancer – Chronic erythroid leukemia
Vigliani and Forni, (1976)	6.38×10^5	10950	0.33*	6.02×10^4	2.58×10^4	Cancer – Leukemia
Infante,(1978)	3.19×10^4	3650	0.33*	3.01×10^3	4.29×10^2	Cancer – Leukemia

*0.33d/d (8h/d) is assumed to be equivalent occupational exposure

LOAEL – Lowest Observed Adverse Effects Levels

ED – Exposure Duration (day)

EF – Exposure length (day/day)

LOAEL–ADD - Lowest Observed Adverse Effects Average Daily Dose

LOAEL–LADD - Lowest Observed Adverse Effects Lifetime Average Daily Dose

Table 3 Reported toxicity data for short - term exposure to benzene from animal experiments

LETHAL EFFECTS						
Reference	Test Animal	LOAEL ($\mu\text{g}/\text{m}^3$)	ED (d)	EF (d/d)	LOAEL-HEADD ($\mu\text{g}/\text{kg}/\text{day}$)	Observed Adverse Health Effects
Smyth et al. 1962	Rat	5.1×10^7	1	0.16	2.7×10^7	Death
Carpenter et al. 1944	Rabbit	1.43×10^8	1	0.02	4.1×10^7	Death
Drew and Fouts (1974)	Rat	4.37×10^7	1	0.16	2.4×10^7	Death
NON – LETHAL EFFECTS						
Reference	Test Animal	LOAEL ($\mu\text{g}/\text{m}^3$)	ED (d)	EF (d/d)	LOAEL-HEADD ($\mu\text{g}/\text{kg}/\text{day}$)	Observed Adverse Health Effects
Robinson et al. (1997)	Rat	1.27×10^7	28	0.25	4.29×10^4	Reduction in total splenic cells
Dow (1992)	Rat	1.59×10^6	21	0.25	5.36×10^4	Decreased myeloid and lymphoid cells
Kuna and Kapp (1981)	Rat	1.59×10^5	14	0.25	5.36×10^3	Decreased maternal body weigh
chertkov et al. (1992)	Mice	9.57×10^5	14	0.25	3.36×10^4	Decreased in bone marrow cellularity and leukocytes
Aoyama (1986)	Mice	1.53×10^5	14	0.25	5.38×10^3	Decreased leukocytes count
Li et al. 1986	Rat	3.19×10^5	7	0.33	1.41×10^4	Decreased leukocytes count
Ward et al. (1985)	Mouse	9.57×10^5	14	0.25	3.36×10^4	Decreased lymphocytes and leukocytes
Cronkite et al. 1985	Mouse	3.19×10^5	14	0.25	1.12×10^4	Decreased hematocrit, hemolytic anemia)
Dempster and Snyder (1991)	Mouse	3.19×10^4	7	0.25	1.12×10^3	Decrease in CFU-E numbers
Wells et al., (1991)	Mouse	9.57×10^5	7	0.25	3.36×10^4	Decrease in WBC count
Plappert et al. (1994a)	Mouse	9.57×10^5	7	0.25	3.36×10^4	Increased in lymphocytes
Keller and Snyder 1988	Mouse	6.38×10^4	21	0.25	2.24×10^3	Decreased circulating erythroid precursors
Green et al. 1978	Rat	3.19×10^5	21	0.25	1.07×10^4	Increased incidence of missing sternebrae)
Coate et al. 1984	Rat	3.19×10^5	21	0.25	1.07×10^4	Decreased fetal weight)
Neun et al. 1992	Mouse	9.57×10^5	15	0.25	3.36×10^4	Reduced bone marrow cellularity)
Gill et al. 1980	Mouse	3.19×10^5	8	0.29	4.48×10^4	leukopenia; decrease in marrow cellularity)

LOAEL – Lowest Observed Adverse Effects Levels

ED – Exposure Duration (day)

EF – Exposure length (day/day)

LOAEL-HEADD – Lowest Observed Adverse Effects Level. Human Equivalent Average Daily Dose

Table 4 Reported toxicity data for medium – term exposure to benzene from animal experiments

LETHAL EFFECTS						
Reference	Test Animal	LOAEL ($\mu\text{g}/\text{m}^3$)	ED (d)	EF (d/d)	LOAEL-HEADD ($\mu\text{g}/\text{kg}/\text{day}$)	Observed Adverse Health Effects
Maltoni et al. 1985	Rat	6.38×10^5	105	0.29	2.49×10^4	Death
Cronkite 1986	Mouse	9.57×10^5	112	0.25	3.36×10^4	Death
Rosenthal and Snyder 1987	Mouse	3.19×10^5	140	0.25	1.12×10^4	Death
Cronkite et al 1989	Mouse	9.57×10^5	112	0.25	3.36×10^4	Death
Farris et al, 1993	Mouse	9.57×10^5	112	0.25	3.36×10^4	Death
Cronkite et al. (1985)	Mouse	9.57×10^5	112	0.25	3.38×10^4	Death
Snyder et al. 1982	Mouse	2.94×10^5	35	0.25	1.03×10^4	Death
NON-LETHAL EFFECTS						
Reference	Test Animal	LOAEL ($\mu\text{g}/\text{m}^3$)	ED (d)	EF (d/d)	LOAEL-HEADD ($\mu\text{g}/\text{kg}/\text{day}$)	Observed Adverse Health Effects
Luke et al. 1988b	Mouse	9.57×10^5	91	0.25	3.36×10^4	Depressed rate of erythropoiesis
Ward et al. (1985)	Rat	9.57×10^5	91	0.25	3.21×10^5	Lower cellularity in bone marrow
Green et al. (1981a)	Mouse	3.01×10^4	70	0.25	1.06×10^3	Some increase in spleen weight and cellularity
Ferris et al. (1997)	Mice	3.19×10^5	56	0.25	1.12×10^4	Altered bone marrow cells, and blood cells.
Ward et al. (1985)	Mouse	3.14×10^3	91	0.25	1.10×10^3	Decreased blood cell counts
Cronkite et al. (1985)	Mouse	3.14×10^3	91	0.25	1.10×10^3	Reduced bone marrow cellularity
Plappert et al. (1994a)	Mouse	9.57×10^5	70	0.25	3.36×10^4	Decreased erythrocyte count and slight anemia
Ferris et al. (1993)	Mice	9.57×10^5	112	0.25	3.36×10^4	Developed lymphoid, preputial and lung tumors
Cronkite et al. (1985)	Mouse	9.57×10^5	112	0.25	3.36×10^4	Thymic lymphomas, leukaemia, lymphomas
Cronkite et al. 1989	Mouse	1.39×10^7	112	0.25	4.88×10^5	Decreased stem cells in bone marrow

LOAEL – Lowest Observed Adverse Effects Levels

ED – Exposure Duration (day)

EF – Exposure length (day/day)

LOAEL-HEADD – Lowest Observed Adverse Effects Level. Human Equivalent Average Daily Dose

Table 5. Reported toxicity data for long – term exposure to benzene from animal experiments

NON – CARCINOGENIC EFFECTS							
Reference	Test Animal	LOAEL ($\mu\text{g}/\text{m}^3$)	ED* (d)	EF (d/d)	LOAEL-HEADD ($\mu\text{g}/\text{kg}/\text{day}$)	LOAEL-HELADD ($\mu\text{g}/\text{kg}/\text{day}$)	Observed Adverse Health Effects
Deichmann et al. (1963)	Rat	1.53×10^5	224	0.29	5.97×10^3	8.17	Slight leucopenia was observed
Snyder et al 1982	Mouse	9.57×10^5	222	0.25	2.24×10^4	41.0	Decreased red blood counts and lymphocytes
Snyder et al. (1980)	Mouse	3.19×10^5	490	0.25	1.10×10^4	20.1	Anemia, bone marrow hypoplasia.
Snyder et al. (1980)	Mouse	9.42×10^5	490	0.25	3.31×10^4	606	Bone marrow hyp0plasia
Snyder et al. (1984)	Rat	3.19×10^5	728	0.25	1.06×10^4	145	Decreased lymphocyte counts, splenic hyperplasia
Green et al. (1981a)	Mouse	9.57×10^5	182	0.25	3.36×10^4	615	Reduced red blood cell count, reduced spleen weight
Baarson et al. (1984)	Mouse	1.02×10^5	179	0.25	3.59×10^3	6.56	Depression in the number of splenic nucleated cells and lymphocytes.
Green et al. (1981a)	Mouse	9.57×10^5	182	0.25	3.36×10^4	615	Lymphocytopenia, reduced red blood cell
Deichmann et al. (1963)	Rat	4.78×10^5	224	0.29	1.86×10^4	255	Slight leucopenia
CARCINOGENIC EFFECTS							
Reference	Test Animal	LOAEL ($\mu\text{g}/\text{m}^3$)	ED (d)	EF (d/d)	LOAEL-HEADD ($\mu\text{g}/\text{kg}/\text{day}$)	LOAEL-HELADD ($\mu\text{g}/\text{kg}/\text{day}$)	Observed Adverse Health Effects
Snyder et al. (1980)	Mouse	9.57×10^5	490	0.25	3.90×10^4	71.0	Leukaemia
Snyder et al. (1978b)	Mouse	3.19×10^5	728	0.25	1.12×10^4	205	Developed myelogenous leukaemia
Snyder et al. (1988)	Mouse	9.57×10^5	728	0.25	3.62×10^4	615	Incidence of leukaemia or lymphomas
Cronkite et al. (1985)	Mouse	9.57×10^5	728	0.25	3.36×10^4	615	Incidence of leukaemia all types
Maltoni et al. 1982a	Rat	6.38×10^5	728	0.29	2.49×10^4	3.41×10^4	Hepatomas
Snyder et al. (1984)	Rat	3.19×10^5	728	0.25	1.07×10^4	1.47×10^2	Incidence of total and malignant tumours
Maltoni et al. 1985	Rat	6.38×10^5	728	0.29	2.49×10^4	341	Hepatomas

LOAEL – Lowest Observed Adverse Effects Levels

ED – Exposure Duration (day)

EF – Exposure Frequency (day/day)

LOAEL-HEADD – Lowest Observable Adverse Effects-Human Equivalent Dose in terms of ADD

LOAEL-HELADD – Lowest Observable Adverse Effects-Human Equivalent Dose in terms of LADD

TSD PLOTS FROM HUMAN EPIDEMIOLOGICAL DATA

Figure 1. TSD plots of LOAEL-LADD using epidemiological data for human exposure to benzene (Table 2) estimated for lifetime exposure of carcinogenic and noncarcinogenic effects.

TSD PLOTS FROM ANIMAL TOXICITY DATA

Nonlethal and Noncarcinogenic Effects

Figure 2. TSD plots for benzene exposure with rats and mice using LOAEL- HEADD data from Tables 3, 4 and 5. A, short term exposure (non – lethal effects); B, medium term exposure (non – lethal effects); C, long – term exposure (non – carcinogenic effects)

TSD PLOTS FROM ANIMAL TOXICITY DATA

Lethal and Carcinogenic Effects

Figure 3. TSD plots for benzene exposure with rats and mice using LOAEL- HEADD data from Tables 3, 4 and 5. A, medium term exposure (lethal effects); B, long – term exposure (carcinogenic effects)

COMPARISON OF TSD PLOTS OF HUMAN AND ANIMAL DATA

Figure 4. Comparison of TSD plots of human and animal data for A, LOAEL-ADD (humans) and B, LOAEL-HEADD (animals), STE; C, LOAEL-ADD (humans) and D, LOAEL-HEADD (animals), LTE; E, LOAEL-LADD (humans) and F, LOAEL-HELADD (animals), Lifetime Exposure (data in Tables 1 to 5)

- An alternative technique was used to set guideline values for exposure to benzene.
- Benzene exposure data from human epidemiology studies were evaluated.
- LOAEL used for plotting toxicant sensitivity distribution curves.
- The derived values were 92 $\mu\text{g/kg/day}$ for LTE and 3.4 $\mu\text{g/kg/day}$ for lifetime exposure.
- This method eliminates the use of safety factors and other sources of errors.