Estimation of the Leukemia Risk in Human Populations Exposed to Benzene from Tobacco Smoke Using Epidemiological Data

Stacy Fiebelkorn* and Clive Meredith

Several epidemiological studies have demonstrated an association between occupational benzene exposure and increased leukemia risk, in particular acute myeloid leukemia (AML). However, there is still uncertainty as to the risk to the general population from exposure to lower environmental levels of benzene. To estimate the excess risk of leukemia from low-dose benzene exposure, various methods for incorporating epidemiological data in quantitative risk assessment were utilized. Tobacco smoke was identified as one of the main potential sources of benzene exposure and was the focus of this exposure assessment, allowing further investigation of the role of benzene in smoking-induced leukemia. Potency estimates for benzene were generated from individual occupational studies and meta-analysis data, and an exposure assessment for two smoking subgroups (light and heavy smokers) carried out. Subsequently, various techniques, including life-table analysis, were then used to evaluate both the excess lifetime risk and the contribution of benzene to smoking-induced leukemia and AML. The excess lifetime risk for smokers was estimated at between two and six additional leukemia deaths in 10,000 and one to three additional AML deaths in 10,000. The contribution of benzene to smoking-induced leukemia was estimated at between 9% and 24% (UCL 14–31%). For AML this contribution was estimated as 11–30% (UCL 22–60%). From the assessments carried out here, it appears there is an increased risk of leukemia from low-level exposure to benzene and that benzene may contribute up to a third of smoking-induced leukemia. Comparable results from using methods with varying degrees of complexity were generated.

KEY WORDS: Benzene; epidemiology; leukemia; tobacco smoke

1. INTRODUCTION

Benzene exposure has been associated with increased incidence of leukemia in humans, in particular acute myeloid leukemia (AML), predominantly based on evidence from occupational epidemiology.\(^1\) The leukemia risk from benzene exposure in the workplace has been documented over the years through a number of occupational studies and follow-ups, including a large industry-based cohort in China and the commonly cited U.S. Pliofilm cohort.\(^2,3\) Accordingly, workplace exposure limits for benzene have been reduced over the last 35 years and the current long-term (8-hour TWA) exposure limit in the United Kingdom is 1 ppm (3.25 mg/m\(^3\)).\(^4,5\) In addition to being widely used in industry, benzene is also found in petrol, exhaust fumes, and tobacco smoke, resulting in the potential for human exposure, not just in occupational settings but also to the wider population. Although the risk of leukemia from

British American Tobacco, R&D Centre, Southampton, SO15 8TL, UK.
*Address correspondence to Stacy Fiebelkorn, British American Tobacco, R&D Centre, Regents Park Road, Southampton SO15 8TL, UK; stacy_fiebelkorn@bat.com.
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occupational exposures to benzene has been well established, the risk to the population from lower environmental levels, such as those from exposure to tobacco smoke, is still not fully understood.\(^{(6)}\)

Tobacco smoke is one of the main potential sources of inhaled benzene exposure for the general population. Several studies have investigated the link between smoking and increased risk of AML, and while these results are not always consistent, there is evidence of a positive association.\(^{(7)}\) and both the International Agency for Research on Cancer and the U.S. Surgeon General have concluded that tobacco smoking causes myeloid leukemia.\(^{(8,9)}\) Two meta-analyses carried out in the last few years have evaluated the available epidemiological data. One of these analyses, by Fircanis \textit{et al.}, identified 17 studies evaluating the relationship between current smokers and AML for inclusion in the meta-analysis, with individual study RRs ranging between 0.93 and 3.47. Eight of the 17 studies reported a significant relationship and the reported overall increased relative risk for current smokers was 1.40 (95% CI, 1.22–1.60).\(^{(10)}\) A second analysis looking at the relationship between smoking and adult myeloid disease (myelodysplastic syndrome and AML) by Wang \textit{et al.} also reported an overall significant increased odds ratio (OR) of similar magnitude (OR 1.45; 95% CI, 1.30–1.62) based on case–control studies only.\(^{(11)}\) The 14 studies included in this analysis reported ORs ranging from 0.93 to 2.00, with 10 out of 14 studies not showing a significant increase in risk from smoking. Although there is clearly some inconsistency in the results from individual studies, both meta-analyses, while using different approaches and study selection criteria, resulted in similar outcomes. Although the overall increased risk for leukemia is not as high as that for other smoking-related diseases such as lung cancer or COPD, and the results are not consistent, these meta-analyses also provide estimates for subgroups of smokers and report an increasing dose–response trend with increased duration and intensity of smoking. This provides further indication of a relationship between smoking and increased risk of AML.

Benzene is present in tobacco smoke at approximately 35 to 80 \(\mu\)g per cigarette for a range of commercial European products selected from Counts \textit{et al.},\(^{(12)}\) which could equate to up to 3 mg/day of benzene for a 40 per day cigarette smoker. Considering the levels of benzene present in tobacco smoke and the clear association with AML incidence, it would not be unreasonable to hypothesize that benzene may be a significant contributor to smoking-induced leukemia. Benzene appears on both the U.S. FDA Harmful and Potentially Harmful Constituents (HPHC) abbreviated list\(^{(13)}\) and the WHO Study Group on Tobacco Product Regulation (TobReg) proposed list for mandatory lowering,\(^{(14)}\) highlighting the importance of further understanding of the low-dose risk and the potential impact of reduced exposure.

The U.S. Plasfilm and China cohorts are two of the most commonly cited cohorts looking at the risk of leukemia from exposure to benzene. Various papers have reported analyses of these cohorts grouped by estimated exposure level, with the lowest exposure groups reported at \(<40\) ppm-years. Reported risk values for this exposure category are 1.45 (95% CI, 0.53–3.31) and 1.9 (95% CI, 0.8–4.7), respectively, for these two studies.\(^{(15,16)}\) Several previous risk assessments for benzene, both qualitative and quantitative, have used data from studies of the U.S. Plasfilm and China cohorts to evaluate the risk of leukemia and AML from exposure to environmental levels of benzene.\(^{(17–19)}\) The quantitative risk assessments utilized various modeling techniques to extrapolate from occupational exposure levels to low-dose exposures. The choice of modeling and extrapolation methods used in quantitative risk assessment can greatly impact the resulting risk estimates. In particular, an understanding of the shape of the dose–response curve is required in order to inform model choice, and to increase confidence in the resulting risk estimates. In the case of benzene, there is some uncertainty as to whether the low-dose response is linear or nonlinear. Benzene is known to require metabolism in order to exert its toxic effects, with several metabolites implicated in its toxicity, including \(\text{tt-muconaldehyde, 1,4-benzoquinone, hydroquinone, and catechol, although there remains uncertainty as to which metabolites are the main contributors to toxicity.}^{(20)}\) A report of more efficient benzene metabolism in humans at concentrations less than 1 ppm\(^{(21)}\) has led to the hypothesis that an additional metabolic pathway operates at low doses and the implication that linear extrapolation from occupational data may therefore underestimate the risk at low dose.\(^{(22)}\) This opens the question of whether a linear model for extrapolation is the most appropriate for benzene; however, there is continuing debate over this hypothesis with two conflicting views being presented in the literature.\(^{(23,24)}\) The U.S. Environmental Protection Agency (EPA) guidelines for carcinogenic risk assessment recommend use of a linear model as a default for extrapolation where
there is no conclusive evidence. The 1998 USEPA assessment for benzene falls on this default assumption and utilizes risk estimates from Crump, extrapolated using a linear model and data from studies based on the U.S. Pliofilm cohort using different exposure estimates. This resulted in a predicted increased risk of $2.2 \times 10^{-6}$ to $7.8 \times 10^{-6}$ (two to eight additional deaths per 1,000,000 exposed) at continuous lifetime exposure to $1 \mu g/m^3$ (0.31 ppb) of benzene in air. A previous study investigating the specific link between smoking-related leukemia and benzene estimated that between 8% and 48% (UCL 20% and 66%) of smoking-induced leukemia is attributable to benzene, again based on data from the U.S. Pliofilm and China cohorts.

The use of epidemiological data is clearly the most relevant for human risk assessment due to the removal of the need for interspecies extrapolation. However, the practicalities of this can be limiting and are generally reliant on the use of human observational studies, predominantly based on occupational exposures in an uncontrolled environment. Not only does this give rise to issues such as confounding factors and inaccurate exposure assessment or quantification, but these studies also tend to be lacking in data at low exposure levels. The methods and guidelines for utilizing animal data in risk assessment have been well characterized but although epidemiology is used where possible, there are no standard methods available. In the case of benzene, there are several occupational studies available and although the U.S. Pliofilm cohort is generally considered most appropriate due to the well characterized cohort, numerous follow-ups, and exposure evaluation, there may be valuable data in additional studies. The use of meta-analysis allows consideration of diverse data sources, which could provide more generalizable results. However, there needs to be an emphasis of study quality and results should be interpreted carefully. One of the aims here is to investigate the use of meta-analyses as part of a quantitative risk assessment and to compare to the results from using a single epidemiological study.

The aim of this study is not only to utilize available data to provide further insight into the risk of leukemia and AML from exposure to low environmental levels of benzene, but also to demonstrate the use of various methods for incorporating epidemiological data as part of a chemical risk assessment, and the impact this can have on resulting risk estimates. In particular, the impact of using different studies and approaches will be evaluated. A complex data intense approach using life-table analysis will be compared against a simplified assessment to estimate the excess lifetime risk (ELR) from exposure to benzene and to evaluate the contribution of benzene to smoking-induced leukemia. The application of these methods to benzene allows the investigation of different risk assessment methods where there are ample data available to demonstrate use of the approaches and assesses the suitability for future application where data are limited.

### 2. METHODS

#### 2.1. Exposure Assessment

An estimate of the daily level of inhaled benzene for two smoking exposure categories selected to represent heavy (>20 cigarettes per day) and light (0–20 cigarettes per day) smokers was generated using published data for benzene levels from various sources, as detailed below. For exposure from environmental sources, time activity data for the average time spent indoors, outdoors, and traveling was used in combinations with average concentrations of benzene measured outdoors in the United Kingdom and in the home (Table I). Exposure to exhaust fumes when traveling by car and refueling was identified as a potentially high

| Source | Daily Exposure | Reference |
|--------|----------------|-----------|
| Air, urban background (21 sites) | 0.64 $\mu g/m^3$ | AEA (31) |
| Air, indoor home (nonsmoker) | 11.5 $\mu g/m^3$ | Kim et al. (32) |
| Air, indoor home (smoker) | 16.3 $\mu g/m^3$ | Kim et al. (32) |
| Refueling | 193.4 $\mu g/m^3$ | Leung & Harrison (33) |
| In vehicle | 203.7 $\mu g/m^3$ | Kim et al. (32) |
| Smoking (light) | 0.649 mg | Counts et al. (12) |
| Smoking (heavy) | 1.947 mg | Counts et al. (12) |
| Food | 0.0005-0.0024 mg | MAFF (52) |
| Water | 0.002 mg | E.U. Directive (53) |

*Daily exposure via drinking water estimated using the E.U. standard (1 $\mu g/L$) as a maximum benzene concentration and a daily consumption of 2L.
source of benzene exposure; therefore, travel was considered to be by car with one minute refueling per day included. Average in-car \( \mu \text{g per 1R4F Kentucky reference cigarette was used.}^{(12)} \) Light smokers were assumed to smoke 10 cigarettes per day and heavy smokers 30 cigarettes per day. Due to the variable nature of human smoking behavior, there is no single representative machine cigarette smoking regime. However, a previous study indicated that the Massachusetts smoking regime produces yield values nearer the average human exposure than alternative regimes such as the International Organization for Standardization and Health Canada Intense regimes, which would be closer to the lower and upper limits, respectively.\(^{(34)} \) Exposure to environmental tobacco smoke (ETS) was captured by using the difference between the concentration of benzene measured in the homes of smokers and those of nonsmokers. Only sources of inhaled benzene were included in this assessment, although estimates of oral exposure levels were at approximately 5 \( \mu \text{g/day} \) (less than 2% of total exposure; Table I). Exposures were averaged over a day and estimated based on an assumed human daily breathing volume of 20 m\(^3\).\(^{(35)} \)

2.2. Study Identification

Two meta-analyses for benzene exposure and leukemia risk were identified where the available cohort and case–control studies with quantitative benzene exposure assessments were evaluated and combined as appropriate to provide dose–response estimates.\(^{(36,37)} \) Only one of these studies investigated leukemia subtypes including AML.\(^{(37)} \) Although AML has been specifically associated with benzene exposure, limiting analysis to a single subtype can reduce the statistical power to detect differences in risk between exposure groups and reduces the number of studies that can be included in analysis. Therefore, both total leukemia and AML endpoints have been included here in all further assessments.

The Khalade \( et \ al. \)\(^{(37)} \) meta-analysis identified nine studies of leukemia risk with cumulative benzene exposure estimates. Using a meta-regression model and average cumulative exposure, the summary effect estimates for low (\(<40 \text{ ppm-years})\), medium (40–99.9 \text{ ppm-years}) and high (\(>100 \text{ ppm-years}) dose groups were 1.64 (95% CI, 1.13–2.39), 1.90 (95% CI, 1.26–2.89), and 2.62 (95% CI, 1.57–4.39), respectively. Study risk estimates for leukemia were heterogeneous, but when grouped by average cumulative exposure levels this was greatly reduced. Only four studies were identified for the AML analyses and the data were considered homogenous. Summary effects reported were as follows: low: 1.94 (95% CI, 0.95–3.95); medium: 2.32 (95% CI, 0.90–5.94); and high: 3.20 (95% CI, 1.09–9.45).

The study by Vlaanderen \( et \ al. \)\(^{(36)} \) only analyzed total leukemia, not specific subtypes, stating that this would be “hampered by a lack of data.” They identified nine studies suitable for analysis; seven of these were also used in the Khalade \( et \ al. \)\(^{(37)} \) analysis. Vlaanderen \( et \ al. \)\(^{(36)} \) used both linear and natural spline models and suggested that the natural model was a slightly better fit based on values for model deviance. The models were then used to predict the relative risk of leukemia for three cumulative exposure levels and a sensitivity analysis was carried out to determine the impact of study types and individual studies. Using the natural spline model without intercept based on all study data, the predicted relative risks for three exposure levels were as follows: 10 ppm-years: 1.22 (95% CI, 1.11–1.34); 20 ppm-years: 1.46 (95% CI, 1.22–1.75); and 40 ppm-years: 1.96 (95% CI, 1.44–2.68).

The two meta-analyses employed different quality assessment criteria and methods, resulting in slightly different study sets included in each analysis, and in risk estimates for different exposure levels. For this reason, and to assess the impact of these differences in the overall assessment, both of these analyses have been used to estimate the potency of benzene. As a comparison, data from the U.S. Pliofilm and China cohorts were also considered here as individual studies to generate potency estimates.\(^{(15,16)} \) Data from these two cohorts were included in the study sets used for both meta-analyses.

2.3. Potency Estimation

A potency value for benzene was estimated from epidemiological data using previously published methods.\(^{(38)} \) The method involves the use of an underlying linear model: \( RR = 1 + \beta \times \text{dose} \), where \( RR \) is the relative risk and \( \beta \) is the potency. Carcinogenic potency and associated confidence limits were estimated by fitting a trendline to risk estimates from the two meta-analyses. Chi-squared goodness-of-fit values for potency estimate models were generated where possible, using observed and expected number.
of cases for each data set. The acceptance level was set at \( p > 0.05 \). Cumulative exposure in ppm-years was used as the dose metric because this is the most commonly reported unit and could be used as an approximation of lifetime exposure. This was converted to cumulative milligrams exposure based on a 240-day working year and 10 m\(^3\) workday breathing volume.\(^{35,39, p. 16}\)

### 2.4. Excess Lifetime Risk

An estimate of the excess risk (ER) from exposure to benzene can be obtained by multiplying the potency estimates generated in Section 2.3 (risk per cumulative mg) by the estimated cumulative exposure to benzene. By assuming that the background risk is 1, the relative risk (RR) for the given level of benzene exposure becomes \( \text{ER} + 1 \). To estimate the absolute ELR for a specific population, a measure of the background risk can be utilized as previously described.\(^{38}\)

\[
\text{ELR} = \text{ER} \times \text{background risk.}
\]

The background risk of leukemia and AML was estimated for males and females based on gender and age-specific mortality rates for 2012 in England and Wales.\(^{40,41}\) Life tables were used to generate the background risk estimates and included information for five-year age groups from age 0 to 75 (truncated at age 75 to avoid instability in the older age groups). Mortality rates have been used to determine the probability of death from all causes in each age group using the formula, \( \Pr = 1 - e^{-\lambda t} \), where \( \Pr \) is the probability of death, \( \lambda \) is the mortality rate, and \( t \) is time in years. The leukemia, AML, and all-cause mortality rates can then be used for each age band to estimate the probability of death from leukemia, conditional on survival to that age band. From this, the cumulative probability of death up to age 75 can also be calculated. The background risk of leukemia mortality was estimated as 0.343% for males and 0.225% for females (34.3 and 22.5 expected deaths in 10,000, respectively). For AML it was estimated as 0.195% for males and 0.140% for females (19.5 and 14 deaths in 10,000, respectively). The following analyses are based on the background risk and mortality rates for males only.

In addition to the method above, the ELR was also estimated using a more complex process based on life tables (as described above for background risk estimation), with the leukemia or AML mortality rates adjusted for benzene exposure by multiplying by \((1 + \beta \times \text{dose})\). Dose was calculated for each age group based on cumulative exposure, with exposure from smoking beginning at age 20. This resulted in slightly different total cumulative exposures than those reported in Table III (25,880 and 50,770 mg for light and heavy smokers, respectively). The background risk was then subtracted from the adjusted lifetime risk for each exposure category to give the ELR. The ELR is presented here as an absolute estimate of the additional number of leukemia deaths (per 10,000) over the background mortality rate and was estimated for the two smoking exposure scenarios (heavy and light) as previously detailed in Section 2.1, using both of the methods described above.

### 2.5. Benzene Contribution

To further understand the impact of benzene exposure from tobacco smoking in particular, the contribution of benzene to smoking-induced leukemia and AML was estimated. By directly comparing a published leukemia relative risk estimate for a specific smoking category, with the predicted ER for the level of benzene exposure associated with that smoking category, the risk attributable to benzene can be estimated.

\[
\text{Contribution of benzene (\%)} = \left( \frac{\text{ER}}{\text{RR}_{\text{pub}} - 1} \right) \times 100,
\]

where ER is the estimated ER based on cumulative benzene exposure (60 years) for light and heavy smokers as previously detailed (including exposure from ETS), and \( \text{RR}_{\text{pub}} \) is a published relative risk for leukemia or AML for the specified smoking exposure scenario (Table II).

Life tables were also used to estimate benzene contribution to allow a more detailed consideration of age-specific mortality rates for different smoking categories, former, never, light, and heavy smokers. All-cause, leukemia, and AML mortality rates were partitioned for each smoking category using age-specific smoking prevalence data\(^{42}\) and relative risks as described in Hertz-Picciotto and Hu.\(^{43}\) Relative risks were taken from published epidemiological studies and can be found in Table II.\(^{44-46}\) The values presented here are for males, with the exception of AML, for which gender-specific relative risks were not available.

To estimate the risk of leukemia from smoking attributable to benzene, similar methods to those used in Korte et al.\(^{26}\) were applied; however, the
ER from smoking was estimated against a background risk of smokers assuming no increased risk of leukemia or AML (using nonsmoker mortality rates). The ER due to benzene (benzene effect) was calculated by dividing the mortality rates for each exposure category by \((1 + \beta \times \text{dose})\) and subtracting the resulting cumulative risk from the total lifetime risk. The attributable fraction was then calculated as the benzene effect divided by the ER from smoking.

### 3. RESULTS

#### 3.1. Exposure Assessment

The daily exposure to benzene for smokers was estimated at between 0.06 mg/m\(^3\) (0.02 ppm) and 0.125 mg/m\(^3\) (0.04 ppm). By removing the benzene exposure associated with tobacco smoke (direct consumption and ETS), nonsmoker exposure can be estimated as approximately 460 \(\mu\)g/day (0.023 mg/m\(^3\)). Therefore, a potential 80% of smokers’ exposure to benzene is attributable to tobacco smoke. For ELR calculations, exposure estimates from all inhalation sources identified here were used (Table III). For investigating the benzene contribution to smoking-induced leukemia, this was adjusted to include only the daily benzene exposure attributable to smoking (light smokers: 740 \(\mu\)g; heavy smokers: 2,040 \(\mu\)g).

#### 3.2. Potency Estimation

The potency estimates for leukemia, based on the two meta-analyses, gave a range of \(1.8 \times 10^{-6}\) to \(3.0 \times 10^{-6}\) per cumulative milligram. The two individual study estimates for the China and Pliofilm cohorts fell within this range. For AML, the potency estimates were similar and ranged between \(2.6 \times 10^{-6}\) and \(3.3 \times 10^{-6}\) per cumulative milligram. All the potency estimates can be found in Table IV.

#### 3.4. Excess Lifetime Risk

The estimated RR for exposure to benzene in tobacco smoke range from 1.03 to 1.17 and are summarized in Table V. The RRs for total benzene exposure were used in conjunction with the estimated background risk for the selected population (England and Wales) to generate the ELR. The ELR of leukemia and AML from exposure to benzene were estimated using the two methods described above (Table VI). For smokers, the initial ER estimates were higher than those estimated using the life-table method and ranged from approximately 2 to 6 in 10,000 additional leukemia deaths and from 1 to 3 in 10,000 for AML.

#### 3.5. Benzene Contribution

The contribution of benzene to both smoking-induced leukemia and AML was also estimated using two methods, both of which gave similar results. For leukemia these were between 9% and 24% (UCL 14–31%) and between 11% and 30% (UCL 22–60%) for AML. The various contributions for the different potency estimates, exposure categories, and assessment methods can be seen in Table VII.

### 4. DISCUSSION

To utilize the epidemiological data for benzene as part of a quantitative risk assessment, a simple linear model has allowed the estimation of benzene potency from occupational data and subsequent extrapolation to low-dose exposure. Potency estimates for benzene were generated using data from two published meta-analyses, both providing combined risk estimates for leukemia (\(1.8 \times 10^{-6}\) [UCL \(2.8 \times 10^{-6}\])] and \(3.0 \times 10^{-6}\) [UCL \(4.0 \times 10^{-6}\)] per cumulative milligram benzene), with only one reporting data for AML specifically (\(2.6 \times 10^{-6}\) [UCL \(5.2 \times 10^{-6}\)])
Table III. Summary of Estimated Daily Inhaled Benzene Concentration for Three Exposure Categories

| Exposure Category | Indoor | Travel | Outdoor | Smoking | Total | Average Daily mg/m³ | 60 Years Cumulative mg |
|-------------------|--------|--------|---------|---------|-------|---------------------|------------------------|
| Nonsmoker         | 211.8  | 247.1  | 0.24    | 0       | 459.2 | 0.023               | 10060                  |
| Light smoker      | 300.2  | 247.1  | 0.24    | 649     | 1197  | 0.060               | 26220                  |
| Heavy smoker      | 300.2  | 247.1  | 0.24    | 1947    | 2495  | 0.125               | 54670                  |

Table IV. Benzene Potency Estimates for Leukemia and AML

| Study                  | Endpoint | Potency per Cumulative Millgram | p-Value |
|------------------------|----------|---------------------------------|---------|
| Vlaanderen et al. (36) | Leukemia | $3.0 \times 10^{-6} (4.0 \times 10^{-6})^a$ | N/A     |
| Khalade et al. (37)    | Leukemia | $1.8 \times 10^{-6} (2.8 \times 10^{-6})$ | 0.22    |
| Hayes et al. (15)      | Leukemia | $2.1 \times 10^{-6} (4.1 \times 10^{-6})$ | 0.40    |
| Rinsky et al. (16)     | Leukemia | $2.6 \times 10^{-6} (4.9 \times 10^{-6})$ | 0.73    |
| Khalade et al. (37)    | AML      | $2.6 \times 10^{-6} (5.2 \times 10^{-6})$ | 0.55    |
| Hayes et al. (15)      | AML      | $3.3 \times 10^{-6} (7.1 \times 10^{-6})$ | 0.33    |

*aNumbers in parentheses are the upper confidence limits.

Table V. Summary of Relative Risk Estimates for Exposure to Benzene in Tobacco Smoke

| Study                  | Endpoint | Light Smoker | Heavy Smoker |
|------------------------|----------|--------------|--------------|
| Vlaanderen et al. (36) | Leukemia | 1.05 (1.06)  | 1.13 (1.18)  |
| Khalade et al. (37)    | Leukemia | 1.03 (1.04)  | 1.08 (1.12)  |
| Khalade et al. (37)    | AML      | 1.04 (1.08)  | 1.17 (1.23)  |

*aNumbers in parentheses are the upper confidence limits.

Table VI. Excess Lifetime Risk Estimates: Number of Additional Leukemia Deaths per 10,000 for Heavy and Light Smoking Exposure Categories

| Study                  | Endpoint | Light Smoker | Heavy Smoker |
|------------------------|----------|--------------|--------------|
| Vlaanderen et al. (36) | Leukemia | 2.7 (3.6)^a  | 2.2 (2.9)    | 5.6 (7.4) | 4.3 (5.7) |
| Khalade et al. (37)    | Leukemia | 1.6 (2.5)    | 1.3 (2.0)    | 3.4 (5.2) | 2.6 (4.0) |
| Khalade et al. (37)    | AML      | 1.3 (2.6)    | 1.1 (2.2)    | 2.8 (5.5) | 2.2 (4.3) |

*aNumbers in parentheses are the upper confidence limits.

per cumulative millgram benzene). Individual study data for leukemia mortality from the China (15) and U.S. Pliofilm (16) cohorts were also used to generate potencies, both of which fell within the range of the two meta-analyses estimates (Table IV). The Pliofilm and China studies employed different methods, with the Pliofilm study using mortality data and generating standardized mortality ratios (SMRs) using the U.S. population as the comparison group, whereas the China cohort utilizes both mortality and incidence data, with unexposed workers as the referent population. Interestingly, both yield similar potency results. The estimates generated here are slightly higher than previously published leukemia potencies based on linear modeling of data from the same two cohorts ($1.7 \times 10^{-6}$ and $6.6 \times 10^{-7}$ per cumulative millgram, for the China and Pliofilm cohorts, respectively). (26) However, the data used in Ref. 26 for the Pliofilm cohort risk estimate were taken from an earlier publication that included a shorter follow-up period and utilized different exposure assessment methods. (19) Additional potencies published by
Table VII. Estimates of the Contribution of Benzene to Smoking-Induced Leukemia or AML

| Study          | Endpoint | Light Smoker | Heavy Smoker |
|---------------|----------|-------------|--------------|
|               |          | Initial    | Life Table   | Initial    | Life Table   |
| Vlaanderen et al. (36) | Leukemia | 15.0 (20.0)^a | 14.4 (19.0) | 21.8 (28.9) | 23.7 (30.6) |
| Khalade et al. (37)    | Leukemia | 9.1 (14.0) | 8.8 (13.5) | 13.1 (20.2) | 14.9 (22.2) |
| Khalade et al. (37)    | AML      | 30.2 (59.5) | 25.2 (48.2) | 11.0 (21.7) | 15.6 (28.4) |

^aNumbers in parentheses are the upper confidence limits.

Crump (19) for the Pliofilm cohort, equivalent to approximately $1.4 \times 10^{-6}$ and $2.2 \times 10^{-6}$ per cumulative milligram generated from a multiplicative linear model using two different exposure assessments, are closer to our estimates. This demonstrates the differences that can be seen not only between estimates derived from different studies but also from the same cohorts using varying methods, thus highlighting the importance of considered study design and method selection, but also giving some confidence that the methods and studies used here are not generating implausible results and are in line with those previously published.

The two meta-analyses identified from the literature used slightly different inclusion criteria and analysis methods. Both studies identified heterogeneity in the study estimates, likely due to differences in average cumulative exposure levels. (37) One of the limitations of meta-analyses, particularly when there is significant heterogeneity, is the potential of combining studies that are not compatible and leading to uninformative results. This should be considered in the interpretation of results and here the individual study data have also been utilized to aid in this process. The Vlaanderen et al. (36) study employed various models, both linear and nonlinear, and carried out a sensitivity analysis to understand the impact of individual studies on the overall estimate. The nonlinear natural spline model was considered to have a marginally better fit than the linear model and showed a supra-linear response, with a greater increase in leukemia risk at low-level exposures, which would support the theory of more effective benzene metabolism at low-dose exposure. As would be expected, the use of these predicted supra-linear risk estimates resulted in a higher predicted potency than using the Khalade et al. (37) risk estimates. Potencies from both analyses were used in all further assessments to give a range of results as there was no conclusive reasoning to exclude either study. Only the Khalade et al. (37) analysis considered the leukemia subtype AML, and whereas AML has been specifically associated with benzene exposure and would be the most appropriate end-point for risk assessment, these results are based on far less data than that for leukemia. Therefore, confidence in the assessments for AML is reduced and the results should be interpreted as such.

Daily benzene intake from inhaled sources was estimated at 1,200 and 2,500 μg for light and heavy smokers, respectively. A previous exposure assessment by Duarte-Davidson et al. (17) used a value of 522 μg/day for an urban smoker. This is much less than the values used here; however, the Duarte–Davidson values were based on 40 μg benzene per cigarette, 20 cigarettes per day, and using a 50% retention assumption. (17) Our estimates used 64.9 μg per cigarette, included an estimate of exposure to ETS, and were based on inhaled dose rather than retained dose, as the potency estimates used here were generated based on this dose metric and the measured retention of benzene in cigarette smoke is 89–98%. (47) The estimation of benzene exposure from ETS is problematic due to a lack of detailed data regarding both the levels present in sidestream smoke and the amount inhaled by a smoker. Previous estimates have been based on measured levels of benzene in sidestream smoke that range from 300 to 700 μg per cigarette. (26) In this study an alternative approach utilizing measured benzene concentrations in the homes of nonsmokers and smokers was applied to capture potential daily ETS exposure, resulting in an estimate of 88 μg per day from ETS. Although this provides an alternative estimate of average daily ETS exposure, it does not consider peak exposures when smoking and does not account for differences in exposure from varying cigarette consumption. Further development would be needed to address this issue as further data become available.

Although the values used here represent an estimate of the amount of benzene potentially inhaled,
this does not represent internal dose and how this may vary in a population. In particular, the estimate of benzene exposure from ETS is likely to vary depending on smoking status and intensity in the home and other factors such as location, occupation, and travel may also influence environmental exposure levels. A urinary biomarker of benzene exposure, S-phenylmercapturic acid (S-PMA), has been used in a study based in Germany to estimate daily benzene intake for 72 smokers. The mean estimated daily benzene intake (490–690 μg) is considerably lower than that predicted here for light smokers at 10 cigarettes per day. This indicates that the exposure estimates here may be overly conservative. However, the 95th percentile (2,438–2,475 μg daily intake) is much closer to the estimate here for heavy smokers, which seems a reasonable representation of high-level exposure considering mean cigarette consumption for the Schettgen et al. study was reported as 17 per day (ranging from 2 to 30).

Benzene has previously been identified as a priority toxicant for reduction in tobacco smoke. Using the benzene potency and exposure assessments, estimates of the relative risk of AML from exposure to benzene in tobacco smoke were 1.04 and 1.17 for light and heavy smokers, respectively. Comparing these to the published pooled relative risks for AML and smoking discussed earlier, of 1.4 and 1.45, gives an initial indication that benzene may not be the sole contributor to smoking-related leukemia. However, these published RRs are for current smokers versus nonsmokers and not split by categories of smoking intensity. One of the challenges in this kind of analysis is the limited availability of RRs for specific and consistent smoking categories and disease endpoints from which the attributable fraction for benzene exposure can be estimated. In addition, for the more complex life-table analyses, RRs for former smokers as well as the current smoker categories are required, and are not always reported. The RR input was identified as having a large influence on the resulting attributable fraction as part of an evaluation using the upper and lower ranges of each parameter. Thus, one of the limitations here is the lack of suitable published RRs and reliance on study availability.

The ELR of leukemia mortality was estimated at up to 3 in 10,000 additional deaths for light smokers and 6 in 10,000 for heavy smokers, indicating there is an increased risk from lifetime exposure to benzene that is greater for heavy smokers. The two methods used gave similar results, although the risk estimates from the life tables were lower. As the life-table analysis is more detailed, taking into account age-specific and all-cause mortality rates, these risk estimates may be more appropriate. However, we have demonstrated that in the absence of data for life-table analysis, alternative methods can be utilized and would provide a conservative approach as they may slightly overestimate risk. Analyses for females have not been reported here as the majority of data were based on predominantly male cohorts and there was a lack of robust relative risk data for females. Future work could allow a closer look at differences in risk for males and females, particularly as new data become available.

For smokers, up to 80% of benzene exposure is due to tobacco smoke. The role of benzene in smoking-related leukemia was further investigated, resulting in a predicted contribution of benzene to between 9% and 24% of smoking-induced leukemia and 11–30% of AML, with both methods providing similar results. These are lower than previous estimates of 8–48% of smoking-induced leukemia and between 11.5% and 58% for AML which were based on different potency estimates and a higher estimate of cigarette consumption. An attributable fraction here of 100% would indicate that benzene is the sole contributor to smoking-induced leukemia and a fraction above 100% would indicate that the risk associated with benzene in tobacco smoke is less than when exposed to benzene alone, which would be implausible based on prior knowledge of tobacco smoke toxicity. With benzene predicted here to contribute to less than a third of smoking-induced leukemia, this raises the question of other potential active leukemogens in tobacco smoke such as 1,3-butadiene and formaldehyde, which are present at similar levels to benzene. However, 1,3-butadiene has been linked primarily to lymphoid leukemia rather than the myeloid leukemia associated with smoking and the link between formaldehyde and leukemia is controversial, with currently conflicting views as to its carcinogenicity. It may therefore be reasonable to assume that neither of these toxicants would have a contribution as great as benzene. Although over 6,000 constituents have currently been identified in tobacco smoke and it is possible that there may be several other potential leukemogens present, these results introduce the possibility that there are other contributing factors increasing the potency of benzene when acting as part of a complex mixture such as tobacco smoke. Two metabolites
of benzene, hydroquinone and catechol, both implicated in its toxicity are also present in tobacco smoke. Further investigation into the combined effect of these toxicants may be an appropriate starting point for future mixture toxicology research. In addition, the exposure estimate here does not take into consideration that smoking exposure consists of intermittent peak exposures, which may not be directly comparable in terms of risk to a continuous exposure concentration in occupational settings. The use of several methods for estimation of ELR and the contribution of benzene to smoking-induced leukemia allowed the comparison of these techniques and resulting estimates to evaluate their utility for quantitative risk assessment. In both cases the methods gave similar results, suggesting that a simple method could provide a useful initial assessment in the absence of comprehensive data. The use of meta-analysis allows the consideration of a range of data and increased understanding of the trends in the data that may be particularly applicable for low-dose extrapolation. As there is still ongoing debate in this area, further work would be required to understand the shape of the low-dose response curve for benzene. Although a linear model has been utilized here, the use of meta-analysis data have allowed the consideration of multiple data sets in a single risk assessment and one of these utilized a nonlinear model. Although meta-analyses may not always be appropriate, and study quality inclusion criteria should be monitored, this may aid in overcoming some of the difficulties associated with identifying a single most suitable study for quantitative risk assessment. In particular, in cases where study heterogeneity is identified, individual study estimates may also provide important information on specific populations that may be lost in the meta-analysis. The use of meta-analysis data as part of a chemical risk assessment has been demonstrated here and provides results not dissimilar to individual studies and previously published estimates. However, the mechanisms of benzene-induced leukemia are still a current topic, with ongoing research in the field likely to provide further insights that could be applied to enhance future risk assessments.

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