Original research

Benzene exposure and risk of benzene poisoning in Chinese workers

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ABSTRACT

Objectives Benzene is a known haematoxin and leukemogen that can cause benzene poisoning (BP), that is, a persistent reduction in white cell counts that is strongly associated with increased risk of lymphohaematopoietic malignancies. Data are needed on the exposure–response, particularly at low doses and susceptible populations for clinical and regulatory purposes.

Methods In a case-cohort study among 110 631 Chinese workers first employed 1949–1987 and followed up during 1972–1999, we evaluated BP risk according to benzene exposure level and investigated risk modification by subject (sex, attained age) and exposure-related factors (latency, exposure windows, age at first benzene exposure, coexposure to toluene) using excess relative risk and excess absolute risk models.

Results There were 538 BP cases and 909 benzene-exposed referents. The exposure metric with best model fit was cumulative benzene exposure during a 5-year risk window, followed by a 9-month lag period before BP diagnosis. Estimated excess absolute risk of BP at age 60 increased from 0.5% for subjects in the lowest benzene exposure category (>0 to 10 ppm-years) to 5.0% for those in the highest category (>100 ppm-years) compared with unexposed subjects. Increased risks were apparent at low cumulative exposure levels and for workers who were first exposed at <30 years of age.

Conclusions Our data show a clear association between benzene exposure and BP, beginning at low cumulative benzene exposure levels with no threshold, and with higher risks for workers exposed at younger ages. These findings are important because BP has been linked to a strongly increased development of lymphohaematopoietic malignancies.

Key messages

What is already known about this subject?

⇒ Benzene is a known haematotoxin, leukemogen and causal agent in benzene poisoning (BP), that is, persistent reduction in white cell counts with benzene exposure. Little is known about the exposure–response at low exposure levels or about populations that may be at higher risks for developing benzene-related haematotoxicity indicated by BP.

What are the new findings?

⇒ We found estimated benzene exposure levels to be strongly associated with BP incidence, with risk rising steeply at relatively low cumulative benzene exposures. Workers exposed to less than 2 ppm per year over the last 5 years on average were estimated to have an excess BP risk (at age 60) of 0.5% compared with unexposed workers. This excess risk increased to 5.0% for subjects in the highest exposure category (over 20 ppm per year, on average).

How might this impact on policy or clinical practice in the foreseeable future?

⇒ Benzene exposure occurs globally in several industries and occupations in high income countries at levels usually less than 1 ppm, with higher exposures being reported in low-income and middle-income countries. As BP is linked to increased risk of developing haematopoietic and lymphoproliferative malignancies, our observation of an increased probability of BP at relatively low cumulative exposure levels, with younger workers being particularly sensitive, emphasises the need to further reduce occupational and environmental benzene exposure globally.

INTRODUCTION

Benzene exposure occurs globally in several industries and occupations (eg, chemical manufacturing, and many components of the petrochemical industry) with exposures in high-income countries usually less than 1 ppm. Higher exposures are reported in low-income and middle-income countries in, for example, shoemaking, painting and rubber product manufacturing.[1]

Exposure to benzene has been causally linked to acute myeloid leukaemia (AML), and may be associated with other myeloid and lymphoid neoplasms.[1] The primary non-malignant health effect from benzene is toxicity to haematopoietic stem cells, including neutropenia, lymphopenia, pancytopenia and aplastic anaemia. 'Benzene poisoning' (BP), a compensable condition in China, is characterised by a persistent total white cell count (WCC) less than 4000/µL blood. BP's (sub)chronic suppressive effect on the bone marrow requires (long-term)

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treatment,[2 3] and has been linked with a forty-fold increased risk of developing lymphohaematopoietic neoplasms when compared with workers with the same exposures, but without BP.[3] This suggests that BP may be an important intermediate condition for the benzene-related lymphohaematopoietic malignancies and related disorders (HLD) association.

Most of the existing data on the relation between benzene exposure and associated BP is based on findings from case series that generally lack high-quality quantitative exposure data and appropriate exposure controls,[4 5] limiting the study of the (shape) of the exposure–response relationship. Using data from the Chinese Center for Disease Control and Prevention/National Cancer Institute (NCI) Cohort Study of Chinese Benzene Workers, Dosemeci *et al*[3] demonstrated a strong association between benzene exposure and BP occurrence and concluded that estimated exposures could be used to evaluate the association between benzene exposure and hematopoietic and lymphoproliferative malignancies and related disorders (HLD) risk. Questions remain regarding the shape of the exposure–response relation for benzene and BP, whether a threshold exposure level exists and whether risks may be affected by age, gender or other temporal and demographic factors. Broadening understanding of the benzene exposure-related risk of BP may clarify the exposure–response relationship, particular at low doses and pathogenesis of benzene-induced toxicity and HLDs.

METHODS

Study design

We previously compared cancer risks in 74 827 benzene exposed and 35 804 unexposed workers employed for at least 1 month in several industries during 1972–1987 in 12 Chinese cities.[6] Exposure status was ascertained from 1949 to 1999 based on factory records on use of benzene-containing materials, job information, industrial processes and measurements. The first (1972–1987) and second (1988–1999) follow-up of the cohort used salary and factory health records to identify and follow employed and retired workers. Details of the study design and population follow-up have been described previously[6–8] and are provided in more detail in online supplemental appendix.

For the current analysis, a case-cohort approach was chosen to allow more detailed exposure and confounder assessment. Eligible workers had to have been employed for at least 1 month during 1972–1987. The reference sample (1100 benzene-exposed and 400 unexposed workers) was selected by stratified random sampling, with strata defined based on age at entry, sex and exposure status (defined as working in a factory with potential benzene exposure). A table listing sampling fractions is provided in online supplemental table A1. Cases and subjects in the reference sample were from 545 factories, of which 501 (92%) with potential benzene exposure based on expert assessment.

BP diagnosis

Identification of BP cases was generally through written factory records supplemented by collection of available medical reports and related materials including official certificates of BP diagnosis (ie, required to obtain worker compensation) and available WCC at or before BP diagnosis. Based on physician assessment, all cases were assigned a certainty and severity score. A description of the algorithm used to assign these scores is provided in online supplemental appendix. Sixteen cases where the certainty score indicated that they were unlikely to be BP cases were excluded from further analyses.

Exposure assessment

Factory records were the primary source for historical benzene, toluene, and xylene air measurements, production processes and job histories. Details are described elsewhere[8] and in online supplemental appendix. Industrial hygienists and factory managers oversaw abstraction of historical benzene, toluene and xylene air measurements (primarily short-term, ambient and routinely collected before 2004); production processes and tasks; and work histories. Complete job history was abstracted for each subject from all study factories.

To estimate individual monthly benzene, toluene, xylene exposures, a statistical model was built from the historic routine monitoring data. The Bayesian hierarchical model allowed for clustering of measurements by factory, workshop, job and date.[8] Non-linear and industry-specific time-trends and predictor effects on a measurement, job and workshop level were incorporated using regression splines and random effects for benzene, toluene and xylene. A 2004–2005 survey on a sample of factories revealed moderate correlation of model predictions with full-shift personal measurements, although with potential underestimation of benzene exposures below 3 ppm.[8] We did not include xylene in the current risk analyses as xylene does not compete with benzene for enzymatic processes and is not a haematotoxin.

Selection of the final study population

A diagram providing an overview of the case-cohort design and subsequent exclusions is provided in online supplemental figure A1. Most notably, we excluded cases and referents from ChongQing because case identification was based on a different (less stringent) definition of BP than in the other study centres and from Guangzhou because case ascertainment was incomplete. We additionally excluded 27 cases because of unknown BP diagnosis date, 35 cases because of a BP diagnosis date before cohort entry and 10 cases for which the date of BP diagnosis was preceded by a diagnosis of HLD. The analytical dataset consisted of 909 exposed workers from the reference sample, and 554 BP cases of which 3 had been randomly selected from the entire population for inclusion in the reference sample.

BP incidence

We evaluated the incidence of BP in the cohort for each year of follow-up to investigate time trends and as a quality check. BP incidence was estimated as the ratio of the count of incident cases of BP over person-time of follow-up in the full cohort based on data from the reference sample, the sampling design and sampling fractions.

Statistical analysis

Cox proportional hazards regression was used to investigate the exposure–response relation between benzene exposure and BP incidence, with attained age as the time-scale and weighting the likelihood function by the inverse sampling fractions to account for the case-cohort design.[9] Cases of BP occurring outside the reference sample were included in their own risk-sets only (ie, the risk-set containing the case subject and all subjects in the reference sample that were at risk for BP at the age the case was diagnosed with BP).[9] Analyses were stratified on sex and adjusted for calendar year (1988–1999 vs 1972–1987) to allow for potential changes in case identification and ascertainment between initial and extended follow-up.

Excess relative risk (ERR) models were fitted using the PEANUTS module in Epicure,[10] expressing the rate ratio (RR) of BP as a linear function of exposure: $RR=1+\beta z$, where βz

is the excess RR (ERR=RR–1) at exposure z, z is a continuous measure of exposure (cumulative exposure in ppm-years or average intensity in ppm) and β is the ERR expressed per unit of exposure.[11] We evaluated different cumulative exposure metrics to find the one that best fitted the data by comparing the Akaike information criterion from ERR models fitted across a grid of combinations of lag periods (0, 1, 3, 6, 9, 12 months) and exposure windows (3, 6 months, 1, 2, 5, 10 years). We then investigated the shape of the exposure–response curve by exploring several non-linear model structures. We evaluated potential effect modification by sex, age at diagnosis, age at first exposure, and coexposure to toluene by estimating the ERR per unit of exposure (β) categories for these variables. We performed sensitivity analyses with inclusion of specific study centres and industries and focusing the analyses on subsets of workers with better quality data.

We estimated excess absolute risks (EAR) for impact assessment by fitting additive hazard models (aalen function, package timereg, R V.3.5.0).[12] In additive hazard models the excess rate (ER) of BP is expressed as a, potential time-varying, function of exposure, ER=α(t)z, where α(t)z is the excess rate at time t for exposure z. The EAR at time t is then estimated as the 'cumulative hazard' $EAR(t) = \int_0^t \alpha(s)\, z ds$.

A more detailed description of the statistical models as well as the time-window analyses to select a suitable exposure metric, the non-linear exposure–response analyses, and the additional sensitivity analyses are provided in online supplemental appendix.

RESULTS

Incidence of BP and subject characteristics of BP cases and referents

We identified a total of 554 BP cases where the certainty of the diagnosis was coded 'definite' (n=529), 'probable' (n=23) or 'possible' (n=2). A further breakdown by BP severity score and materials supporting the diagnosis is provided in table 1 and online supplementla appendix. Only 6% (n=33) were considered to have severe BP (WCC at the time of diagnosis was <2500 cells/μL).

Table 1 Certainty of BP diagnosis based on case severity and diagnostic information

	Certainty of diagnosis		
	Definite n (%)	Probable n (%)	Possible n (%)
Total	529	23	2
Severity			
Severe	32 (6)	1 (4)	–
Moderate	239 (45)	–	–
Mild	114 (22)	–	–
Indeterminate	144 (27)	22 (96)	2 (100)
Basis for BP diagnosis			
Certificate+WCC	69 (13)	–	–
Certificate only	39 (7)	–	–
WCC count only	288 (54)	–	–
Other*	133 (25)	23 (100)	2 (100)

Certainty of diagnosis was primarily based on whether a BP certificate or WCCs were available. See online supplemental appendix for further description and category definitions.
*See online supplemental appendix 1 for a detailed description of the BP case ascertainment and definitions for certainty of diagnosis and severity of BP.
BP, benzene poisoning; WCC, white cell count.

Low incidence rates for BP in the first 2 years of follow-up (1972–1973, online supplemental figure A2A) suggested possible incomplete case ascertainment, thus, we excluded the first 2 years of follow-up (n=13 BP cases) from all further analyses. BP incidence declined progressively over the period 1974–1999, with an estimated incidence of 0.92 cases/1000 person-years in 1974 and an incidence of 0.03 cases/1000 person-years in 1999 (online supplemental figure A2B). This coincided with a marked decrease in benzene exposure levels in the benzene-exposed reference sample from an average estimate of 17.8 ppm in 1974 to 0.3 ppm in 1999 (online supplemental figure A3).

As shown in table 2, BP cases tended to be slightly younger, both at entry into the cohort and at first hire into a cohort factory than the benzene-exposed reference sample. The unadjusted BP incidence rate in women was 1.5 times that in men, despite similar age and (assigned) benzene exposure distributions (not shown).

Temporal aspects of the exposure–response relation

The exposure distribution in cases by time to diagnosis features slightly lower exposure levels in the months directly preceding diagnosis, with a clear drop in the upper (80%) quantile from about 7 months onwards (online supplemental figure A4). Time-window analyses showed that cumulative exposure during a 5-year period after a lag of 9 months provided the best fit (online supplementla figure A5). Most of the workers were exposed to benzene levels <10 ppm on average (the equivalent of <50 ppm-years in a 5-year interval) (online supplemental table A2).

In fitting models that included the highest monthly estimated exposure level within a time window we found that a time window of 5 years in combination with a lag period of 9 months also provided the best fit (data not shown). For each combination of lag and window length, ERR models based on the cumulative exposure metric fitted better than models based on the highest exposure metric. Therefore, all subsequent exposure–response analyses used a 9-month lag and a 5-year cumulation window.

Shape of the exposure–response relation

Model fit was similar for all non-linear models and much better than for the linear model (figure 1). Estimated ERR increased strongly at low benzene exposure levels but levelled off at higher exposures. Findings were similar for models fitted using the highest exposure in the 5-year window before diagnosis as the exposure metric (online supplemental figure A6).

Effect modification

There was little evidence of effect modification by sex, attained age or toluene coexposure (likelihood ratio test p>0.5 for all) (table 3). There was some indication for a higher excess risk of BP in workers first exposed before age 30 years (ERR per 100 ppm-years=1.32) compared with workers first exposed at age>30 years (ERR per 100 ppm-years=0.69; likelihood ratio test p<0.05).

Sensitivity analyses

Results of the sensitivity analyses are presented in online supplemental tables A3 and A4. These sensitivity analyses showed general robustness of exposure–response relations by study centre, industry, data quality and case definitions. Slope estimates were somewhat lower after excluding subjects from Shenyang (20% of BP cases) and when excluding workers in the coatings industry (includes spray painting and painting; contributed more than 50% of the cases) (online supplemental table A3).

Table 2 Characteristics of subjects in the reference sample and benzene poisoning (BP) cases in a case-cohort study of Chinese benzene-exposed workers followed-up during 1974–1999

	Reference sample			BP cases
	n (%)	Weighted personyears (×1000)*	Mean exposure (ppm-years)†	n (%)
Full sample	909 (100.0)	1238.5	25.5	541 (100.0)
Sex				
Males	494 (54.3)	623.4	25.1	220 (40.7)
Females	415 (45.7)	615.1	25.9	321 (59.3)
Age at entry to follow-up (years)				
<20	121 (13.3)	215.8	17.6	56 (10.4)
20–29	318 (35.0)	507.4	20.1	201 (37.2)
30–39	220 (24.2)	291.8	36.4	186 (34.4)
40–49	145 (16.0)	164.8	33.4	86 (15.9)
≥50	105 (11.6)	58.7	25.4	12 (2.2)
Age at first exposure/hire (years)				
<20	214 (23.5)	386.3	28.3	185 (34.2)
20–29	377 (41.5)	547.7	24.0	243 (44.9)
30–39	181 (19.9)	204.0	27.0	93 (17.2)
≥40	137 (15.1)	100.4	20.1	20 (3.7)
Birth cohort				
<1920	61 (6.7)	28.1	21.5	2 (0.4)
1920–29	103 (11.3)	94.4	33.1	43 (7.9)
1930–39	173 (19.0)	230.6	37.4	156 (28.8)
1940–49	189 (20.8)	285.9	28.6	139 (25.7)
1950–59	244 (26.8)	391.2	20.7	155 (28.7)
≥1960	139 (15.3)	208.4	14.3	46 (8.5)
Year of entry to follow-up				
1972–79	646 (71.1)	909.0	30.0	456 (84.3)
1980–87	263 (28.9)	329.5	13.2	85 (15.7)
Year of first exposure/hire				
≤1949	5 (0.6)	5.9	54.1	10 (1.8)
1950–59	126 (13.9)	134.4	47.4	112 (20.7)
1960–71	270 (29.7)	385.8	32.8	186 (34.4)
1972–79	243 (26.7)	379.2	20.9	147 (27.2)
1980–87	265 (29.2)	333.2	13.0	86 (15.9)
Highest education				
None	61 (6.7)	51.4	21.5	18 (3.3)
Primary school	561 (61.7)	759.6	26.7	381 (70.4)
Junior/middle school	156 (17.2)	235.0	24.5	87 (16.1)
High school	49 (5.4)	81.1	28.7	18 (3.3)
University	25 (2.8)	35.3	20.5	29 (5.4)
Other/unknown	57 (6.3)	76.0	18.0	8 (1.5)
Study centre				
Shanghai	187 (20.6)	246.4	10.2	69 (12.8)
Tianjin	149 (16.4)	198.4	23.5	41 (7.6)
Chengdu	127 (14.0)	178.1	14.8	34 (6.3)
Harbin	122 (13.4)	168.7	41.8	108 (20.0)
Shenyang	98 (10.8)	133.2	43.9	109 (20.1)
Jinzhou	34 (3.7)	51.7	57.0	14 (2.6)
Zhengzhou	56 (6.2)	77.8	26.9	30 (5.5)
Luoyang	15 (1.7)	18.2	20.8	30 (5.5)
Nanchang	89 (9.8)	123.6	5.1	77 (14.2)
Kaifeng	32 (3.5)	42.6	66.8	29 (5.4)
Percent of lifetime employment in study factories (%)	90.0%			92.9%
Industries				
(Spray) painting	440 (48.4)	587.1	19.6	271 (50.1)
Rubber	56 (6.2)	80.1	74.1	53 (9.8)
Benzene refining	6 (0.7)	8.8	4.6	5 (0.9)

continued

Table 2 continued

	Reference sample			BP cases
	n (%)	Weighted personyears (×1000)*	Mean exposure (ppm-years)†	n (%)
Shoe	121 (13.3)	143.4	20.5	63 (11.6)
Insulation varnishing	91 (10.0)	139.7	30.4	53 (9.8)
Chemical	142 (15.6)	201.9	23.6	72 (13.3)
Other/mixed	53 (5.8)	77.5	27.8	24 (4.4)
Exposure in 5-year window (ppm-years)§				
0	788 (86.7)	328.5	0.0	28 (5.2)‡
>0–10	791 (87.0)	420.1	3.8	120 (22.2)
>10–30	627 (69.0)	239.1	18.3	129 (23.8)
>30–100	447 (49.2)	175.7	54.5	131 (24.2)
>100	211 (23.2)	75.2	213.4	133 (24.6)

*Person-years weighted by the sampling fraction.
†Mean exposure is calculated as the person-time weighted average of benzene exposure in a 5-year interval before case diagnosis dates lagged by 9 months in the reference sample.
‡28 BP cases were not exposed to benzene in the 5-year interval preceding diagnosis according to our exposure assessment.
§The number of subjects in the reference sample corresponds to the number of subjects ever in that category and the proportions therefore do not add up to 100%.

Slope estimates appeared slightly steeper when cases were restricted to those with either a BP certificate or WCC, or when the analysis was restricted to subjects with more complete or recent exposure information (online supplemental table A4). There was no strong relation between benzene exposure quartiles and BP severity (χ^2 test p=0.16). When the analysis was restricted to cases and referents that were first exposed to benzene in the 5-year interval before the case's diagnosis, the ERR per unit of exposure almost doubled.

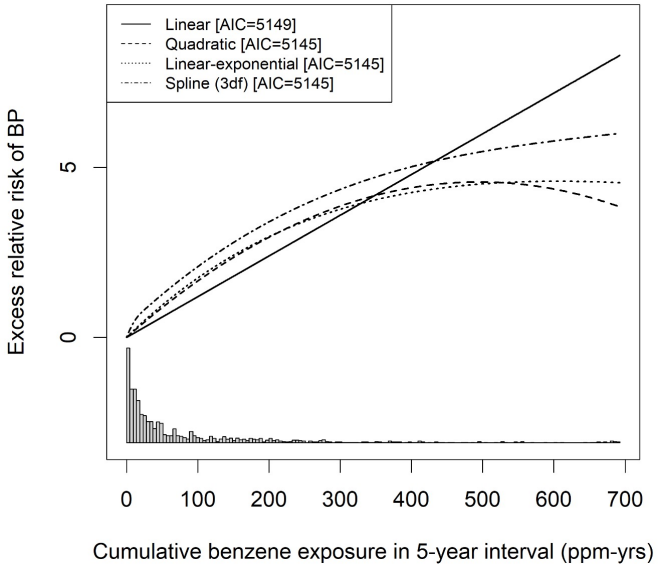

Figure 1 Fitted exposure–response curves for 9 months lagged cumulative benzene exposure in the 5-year interval before age at diagnosis (or matched age for the reference sample) and benzene poisoning (BP) estimated using different exposure–response models. Excess relative risks (RR-1) were estimated from models using baseline risks stratified on sex and adjusted for calendar year (1988–99 vs 1972–87). Person-years of follow-up without benzene exposure in the relevant 5-year interval was considered not at risk for BP and dropped from the analysis. The distribution of the highest estimated cumulative benzene exposures by subject is shown in the rug plot. AIC, Akaike information criterion.

Absolute risks

The EAR for cumulative benzene exposure and risk of BP appeared especially high for those exposed over 100 ppm-years (corresponding to a 5-year average of 20 ppm per year) (figure 2A). Estimated (95% CI) EAR at age 60 years increased from 0.5% (0.1% to 0.9%) for subjects in the lowest exposure quartile (>0 to 10 ppm-years) to 5.0% (3.3% to 7.0%) in the highest quartile (≥100 ppm-years). The EAR of BP at age 60 years based on a regression spline model confirmed that the exposure–response relation was relatively steep at low exposure levels and plateaued at higher benzene exposure levels (figure 2B).

DISCUSSION

This is the largest study to date to explore the exposure–response relation between benzene exposure and BP. Novel aspects include exposure assessment based on a state-of-the-art determinant-based exposure model, rigorous BP case review, and detailed consideration of modification by temporal and subject-specific characteristics. Estimated benzene exposure levels were strongly associated with BP incidence, and risk rose strongly at relatively low cumulative benzene exposures, with no evidence of a threshold, but seemed to level off at higher levels. Workers exposed to less than 2 ppm per year over the last 5 years on average were estimated to have an excess BP risk of 0.5% compared with unexposed workers. This excess risk increased to 5.0% for subjects in the highest exposure category (over 20 ppm per year, on average).

BP incidence was higher in women than in men in this cohort, but without any significant effect modification of the benzene-BP exposure–response relation. Potential explanations for this finding include faster biotransformation of benzene in women,[13] differences in actual (rather than assigned) benzene exposures for men and women, ascertainment bias due to differential health seeking behaviour, and differences in securing a BP diagnosis by gender.

We observed potential effect modification by age at exposure, with a stronger association in workers that had been exposed at a younger age. This is similar to the associations observed in this population for benzene exposure and incidence of myeloid neoplasms (ie, combined myelodysplastic syndromes and AML among those who were under age 25 at first exposure, for

Table 3 Effect modification of the BP relation by subject-related and exposure-related characteristics

	Mean exposure* (ppm-years)	No cases	Person-years (×1000)	ERR per 100 ppm-years (95% CI)	LRT p value†
Full sample‡	38.1	513	13.9	1.20 (0.82 to 1.70)	
Sex					>0.5
Males	39.2	208	7.2	1.15 (0.64 to 1.93)	
Females	36.8	305	6.7	1.23 (0.75 to 1.92)	
Age at diagnosis/attained age (years)					>0.5
<35	30.2	189	5.2	1.31 (0.69 to 2.25)	
35–<40	39.1	96	2.2	1.13 (0.49 to 2.27)	
40–<45	46.4	170	3.8	1.14 (0.60 to 2.03)	
>45	40.6	58	2.7	1.15 (0.26 to 3.27)	
Toluene coexposure					>0.5
No coexposure	–	–	–	–	
Lowest quartile	27.6	129	4.6	1.32 (0.83 to 2.00)	
Second quartile	22.7	128	4.0	1.19 (0.51 to 2.15)	
Third quartile	42.5	128	3.2	0.93 (0.42 to 1.62)	
Highest quartile	84.7	128	2.0	1.19 (0.71 to 1.82)	
Age at first exposure/hire (years)					0.03
<30	35.8	403	10.1	1.32 (0.90 to 1.86)	
≥30	44.1	110	3.8	0.69 (0.25 to 1.32)	

Excess relative risks were estimated from models using baseline risks stratified on sex and adjusted for calendar year (1988–1999 vs 1972–1987).
*Exposure was defined as exposure to benzene within the 5-year interval before age at diagnosis (or matched age for the reference sample) after a lag of 9 months.
†P values are from an LRT of the model including an interaction with the modifying variable compared with the model including only exposure.
‡Full sample statistics are different from those in table 2 due to the restriction to subjects with benzene exposure in the 5-year interval.
BP, benzene poisoning; LRT, likelihood ratio test.

exposure that occurred before age 35, and for workers that were diagnosed before they reached 40 years of age.[7] Haematopoietic stem cells and the stromal microenvironment in younger workers could be more susceptible to the effects of benzene, but this observation needs further investigation as at least one earlier study on HLD (which included only 15 cases) found the opposite.[14]

Cumulative benzene exposure during the 5-year interval preceding BP diagnosis with a lag of 3–9 months was the exposure metric that was most strongly associated with BP. The lag period could reflect the underlying biology of the disease, but it could also reflect the time required to secure a BP diagnosis. The fact that exposures seemed to be lower, especially for the upper quintile of exposure, in the months immediately preceding BP diagnosis suggests that protective measures to reduce benzene exposure may have been implemented as the diagnostic evaluation was underway. We caution against direct causal interpretation of the length of the selected time window (cumulation period) and our finding that a cumulative metric provided a better fit than a metric based on the highest predicted exposure. The time resolution of our exposure assignment was mostly determined by the longer-term temporal pattern of declining

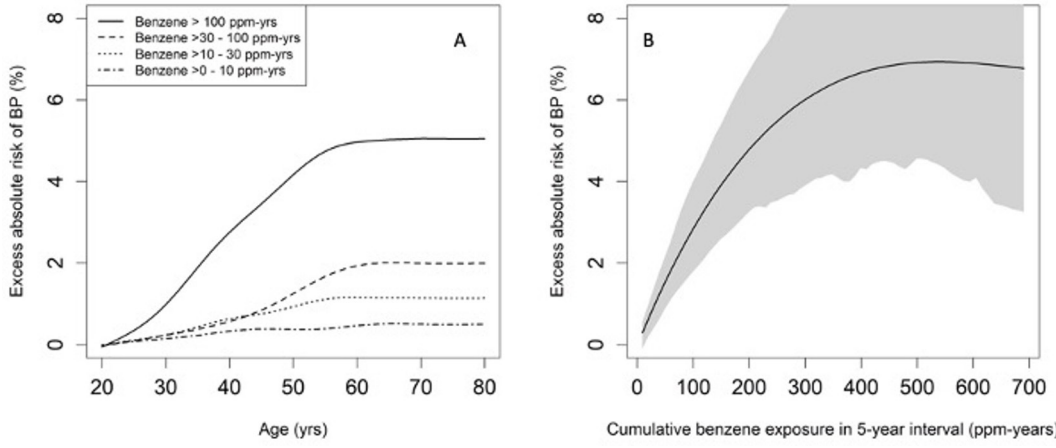

Figure 2 Exposure–response curves for benzene exposure (in categories) and benzene poisoning (BP) based on an absolute-risk model. Estimated excess absolute risks of BP (difference in cumulative risks for exposed and unexposed) as a function of age for subjects within different categories of cumulative benzene exposure (A) or as a function of cumulative benzene exposure for subjects aged 60 years (B) for exposure to benzene within a 5-year window after a lag of 9 months. Excess absolute risks were estimated from additive hazards models using baseline risks stratified on sex and adjusted for calendar year (1988–99 vs 1972–87). In model A, benzene exposure was included as a categorical variable. In model B, a regression spline model with two interior knots (at the 33% and 67% percentiles of exposure) was used.

benzene exposure levels. Ignoring some minor seasonal variation, the highest exposure for any subject during the 5-year interval was therefore likely to be the exposure assigned at the start of the interval, unless there had been a change in job title. Our metric of highest estimated exposure (as an 8-hour average) may therefore correlate only weakly with the occurrence of peak exposure levels.

We observed that the estimated excess relative and absolute risks increased strongly at low benzene exposure levels but levelled off at higher exposures. Similar observations have been made for the exposure–response of benzene and leukaemia[15] and for benzene induced haematoxicity.[16] Such supralinear exposure–response curves could be due to saturation of enzyme systems at the higher end of the exposure range leading to a decrease in formation of toxic benzene-metabolites,[15] depletion of susceptibles in the population[17] or differential exposure misclassification across the exposure range. Our previous exposure validation work,[8] however, did not provide evidence for differential exposure misclassification across the exposure range leaving the first two possibilities as more likely explanations.

Aksoy *et al*[5] were among the first to describe signs of BP (eg, leucopenia, thrombocytopaenia). They observed a clear increase in risk of BP among highly exposed individuals (30–210 ppm; equivalent to a 150–1050 ppm-years range if sustained over a 5-year period). These exposures are at the higher end of our exposure distribution (150 ppm being at the upper 83% quantile of exposures in the reference sample). Our results suggest that BP may occur at much lower benzene exposure levels (10–100 ppm).

Occurrence of BP at relatively low benzene exposures was established from several case series where many cases were diagnosed with BP at average reported concentrations between 10 and 30 ppm,[18 19] but the exposure–response relation over a wide(r) range of exposures was not described. Our results are further strengthened by the cross-sectional observations in benzene-exposed individuals of decreased WCC at low exposure levels (<1 ppm) and threefold increase risk of leucopenia (<4000 cells/μL) for subjects exposed to less than 10 ppm of benzene.[20]

Dosemeci *et al*[3] investigated the BP exposure–response relation in the first follow-up (1972–1987) of our cohort. They found the strongest exposureleucopaeniaresponse relation with exposure levels some 1.5 years before diagnosis. It is difficult to directly compare their results to ours due to differences in the exposure-assessment and the more detailed analytical methods used in the current evaluation (see the Appendix for a more detailed discussion), but the relative risks they estimated are in the same range as ours.

Results from animal studies, although conflicting, have suggested that sex could affect susceptibility to benzene-induced toxicity.[16 21] We found no indication of effect modification by sex. It has also been suggested that coexposure to toluene might inhibit effects of benzene, as both agents compete for the same enzymes and high toluene exposure may therefore reduce production of toxic benzene metabolites. We saw no evidence of effect modification by toluene, similar to our earlier observations of haematological benzene effects in a cross-sectional study.[22]

Strengths of the study

This study is the largest to date, evaluated workers employed in a range of industries and exposure circumstances, and included 46% (benzene-exposed) women, thus enabling assessment of sex-related differences.

Benzene exposure was estimated using a Bayesian hierarchical model, linking data from over 8000 benzene measurements to a variety of factory, workshop and job-specific exposure determinants. This included direct quantitative information for more than 95% of factories where exposed subjects had been employed and for all jobs and all years from 1972 to 1999. Job mobility was relatively low, with most workers having been employed at the same factory throughout follow-up. Fifty-seven per cent of subjects in the reference sample held only one job title, while fewer than 10% held more than two different job titles. The large sample size, high exposure contrast and strong exposure–response relation allowed us to explore a range of different exposure metrics and response shapes, which should be informative for future investigations.

Limitations of the study

As with all observational studies our study could be subject to (unmeasured) confounding. To evaluate the potential for confounding we evaluated demographic, geographic and temporal features and BP case characteristics. Study centre (city) and industry were important predictors of benzene exposure in the exposure model,[8] and incidence rates of BP were associated with study centre and type of industry where subjects worked. Adjustment for study centre and industry, however, had little effect on magnitude or shape of the estimated exposure–response curve.

Incidence rates of BP were lower in the extended follow-up (after 1987) than in the initial follow-up. Much of that decrease is likely due to strong reductions in benzene exposure over the same period; therefore, adjusting for follow-up period may result in overadjustment. Although the slope of the exposure–response relation was reduced by 25% after adjustment for follow-up period, the adjustment had little effect on the shape of the estimated exposure–response curve.

We previously showed, in a 2004–2005 survey of a sample of study factories, that our benzene exposure estimates correlated moderately with full-shift personal measurements, although with potential underestimation of benzene exposures less than 3 ppm. This could have contributed to an amplified supralinearity at the lower end of cumulative benzene-exposure.jo

CONCLUSION

The results of this study show a clear exposure–response relation between estimated benzene exposure and the occurrence of BP in a large cohort of benzene-exposed workers. These effects were observed at relatively low cumulative exposure levels within a 5-year cumulation period. Whether the effects we found are the result of chronic benzene-related toxicity to stem cells and the stromal environment or whether they reflect that 'high' exposure events are more likely to occur at higher average exposures cannot be discerned from our data and require further mechanistic and epidemiological studies. Our findings are important given that BP has been linked to a strongly (40-fold) increased risk for developing HLDs, suggesting that it may be on the causal pathway.

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REFERENCES

1 Loomis D, Guyton KZ, Grosse Y, *et al*. Carcinogenicity of benzene. *Lancet Oncol* 2017;18:1574–5.
2 Rothman N, Smith MT, Hayes RB, *et al*. Benzene poisoning, a risk factor for hematological malignancy, is associated with the NQO1 609C-->T mutation and rapid fractional excretion of chlorzoxazone. *Cancer Res* 1997;57:2839–42.
3 Dosemeci M, Yin SN, Linet M, *et al*. Indirect validation of benzene exposure assessment by association with benzene poisoning. *Environ Health Perspect* 1996;104(Suppl 6):1343–7.
4 Irons RD, Lv L, Gross SA, *et al*. Chronic exposure to benzene results in a unique form of dysplasia. *Leuk Res* 2005;29:1371–80.
5 Aksoy M, Dinçol K, Akgün T, *et al*. Haematological effects of chronic benzene poisoning in 217 workers. *Br J Ind Med* 1971;28:296–302.
6 Linet MS, Yin S-N, Gilbert ES, *et al*. A retrospective cohort study of cause-specific mortality and incidence of hematopoietic malignancies in Chinese benzene-exposed workers. *Int J Cancer* 2015;137:2184–97.
7 Linet MS, Gilbert ES, Vermeulen R, *et al*. Benzene exposure response and risk of myeloid neoplasms in Chinese workers: a multicenter case-cohort study. *J Natl Cancer Inst* 2019;111:465–74.
8 Portengen L, Linet MS, Li G-L, *et al*. Retrospective benzene exposure assessment for a multi-center case-cohort study of benzene-exposed workers in China. *J Expo Sci Environ Epidemiol* 2016;26:334–40.
9 Langholz B, Jiao J. Computational methods for case-cohort studies. *Comp Stat Data Anal*.
10 Preston DL, Shilnikova NS. The EPICURE regression Prgrams. User manual and command summary. 2018.
11 Prentice RL, Mason MW. On the application of linear relative risk regression models. *Biometrics* 1986;42:109–20.
12 Martinussen T, Scheike T. *Dynamic regression models for survival data*. New York: Springer Science, 2006.
13 Brown EA, Shelley ML, Fisher JW. A pharmacokinetic study of occupational and environmental benzene exposure with regard to gender. *Risk Anal* 1998;18:205–13.
14 Richardson DB. Temporal variation in the association between benzene and leukemia mortality. *Environ Health Perspect* 2008;116:370–4.
15 Vlaanderen J, Portengen L, Rappaport SM, *et al*. The impact of saturable metabolism on exposure-response relations in 2 studies of benzene-induced leukemia. *Am J Epidemiol* 2011;174:621–9.
16 Verma Y, Rana SVS. Sex differences in oxidative stress induced by benzene in rats. *Indian J Exp Biol* 2004;42:117–20.
17 Steenland K, Karnes C, Darrow L, *et al*. Attenuation of exposure-response rate ratios at higher exposures. *Epidemiology* 2015;26:395–401.
18 Kuang S, Liang W. Clinical analysis of 43 cases of chronic benzene poisoning. *Chem Biol Interact* 2005;153-154:129–35.
19 Zhao Z, Zhu Z, Zhang S, *et al*. [Professional chronic benzene poisoning in workers exposed to low-concentration benzene: a report of 5 cases]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 2015;33:118–20.
20 Lan Q, Zhang L, Li G, *et al*. Hematotoxicity in workers exposed to low levels of benzene. *Science* 2004;306:1774–6.
21 Corti M, Snyder CA. Gender- and age-specific cytotoxic susceptibility to benzene metabolites in vitro. *Toxicol Sci* 1998;41:42–8.
22 Rappaport SM, Kim S, Lan Q, *et al*. Human benzene metabolism following occupational and environmental exposures. *Chem Biol Interact* 2010;184:189–95.