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# Exposure to benzene and childhood leukaemia: a pilot case-control study

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## ABSTRACT

**Objectives:** *Main purpose* To evaluate the feasibility of a measurement-based assessment of benzene exposure in case-control studies of paediatric cancer; *Additional aims* To identify the sources of exposure variability; to assess the performance of two benzene biomarkers; to verify the occurrence of participation bias; to check whether exposures to benzene and to 50 Hz magnetic fields were correlated, and might exert reciprocal confounding effects.

**Design:** Pilot case-control study of childhood leukaemia and exposure to benzene assessed by repeated seasonal weekly measurements in breathing zone air samples and outside the children's dwellings, with concurrent determinations of cotinine, *t-t*-muconic acid (MA) and sulfo-phenylmercapturic acid (S-PMA) in urine.

**Participants:** 108 cases and 194 controls were eligible for inclusion.

**Results:** Full-participation was obtained from 46 cases and 60 controls, with low dropout rates before four repeats (11% and 17%); an additional 23 cases and 80 controls allowed the collection of outdoor air samples only. The average benzene concentration in personal and outdoor air samples was 3 $\mu g/m^3$ (SD 1.45) and 2.7 $\mu g/m^3$ (SD 1.41), respectively. Personal exposure was strongly influenced by outdoor benzene concentrations, higher in the cold seasons than in warm seasons, and not affected by gender, age, area of residence or caseness. Urinary excretion of S-PMA and personal benzene exposure were well correlated. Outdoor benzene levels were lower among participant controls compared with non-participants, but did not differ between participant and non-participant cases; the direction of the bias was found to depend on the cut-point chosen to distinguish exposed and unexposed. Exposures to benzene and extremely low-frequency magnetic fields were positively correlated.

**Conclusions:** Repeated individual measurements are needed to account for the seasonal variability in benzene exposure, and they have the additional advantage of increasing the study power. Measurement-based assessment of benzene exposure in studies of childhood leukaemia appears feasible, although it is financially and logistically demanding.

## ARTICLE SUMMARY

### Article focus

- Benzene is an established cause of acute non-lymphocytic leukaemia, and there is limited evidence for an association between exposure to this agent and other haematological neoplasms. Epidemiological studies of benzene and childhood leukaemia have provided inconsistent results, possibly due to the use of surrogate exposure proxies, and lack of analyses by leukaemia subtype.
- Our pilot study was aimed at evaluating the logistic feasibility of an assessment of benzene exposure based on repeated measurements in a case-control study of childhood leukaemia. A few methodological issues were also addressed (putative determinants of exposure variability; performance of urinary levels of muconic acid (MA) and sulfo-phenylmercapturic acid (S-PMA) as benzene biomarkers in children; participation bias; possible reciprocal confounding effects of exposures to benzene and to extremely low frequency magnetic fields (ELF-MF)).

### Key messages

- Eligible for inclusion were 108 cases and 194 matched controls, aged 2–12 years at the time of the survey. Full participation rates were low, but the outdoor monitoring was accepted by 64% of cases and 72% of controls. Adherence of full participants to the scheduled repeats was very satisfactory (cases 89%, controls 83%).
- Personal exposure was strongly influenced by outdoor benzene concentrations, was higher in the cold seasons than in warm seasons and was not affected by gender, age, area of residence or caseness. Personal benzene exposure and urinary excretion of S-PMA (but not of MA) were well correlated. A participation bias was indeed present. A positive association between exposures to benzene and ELF-MF was observed.
- Epidemiological studies of paediatric cancer and estimates of environmental benzene exposure based on repeated seasonal measurements, although challenging, appear logistically feasible.

## ARTICLE SUMMARY

### Strengths and limitations of this study

- To our knowledge, this is the first pilot study of childhood leukaemia and measured personal benzene exposure.
- The study size is very small. The greater accuracy of measurement-based exposures estimates, compared with surrogate exposure proxies, does not necessarily correspond to increased validity, especially when measurements are used for retrospective postdiagnosis exposure assessments.

## INTRODUCTION

Benzene is a ubiquitous air pollutant that needs to be metabolised to become carcinogenic.[1 2]

Benzene exposure and acute non-lymphocytic leukaemia (AnLL) are causally related in adults, while there is limited evidence for an association between exposure to this agent and acute or chronic lymphocytic leukaemia, multiple myeloma and non-Hodgkin's lymphoma.[3] Moreover, a dose-dependent association between benzene exposure and incidence of myelodysplastic syndrome has been observed among petroleum workers.[4]

Exposure to benzene would increase the risk of AnLL at levels of ≥40 ppm-years of occupational cumulative exposure, equivalent to a lifetime (76 years) environmental exposure of ≥120 ppb.[5]

Owing to the established carcinogenicity of benzene, WHO has not developed any guideline value for this chemical in air, while indicating that ambient benzene concentrations of 17, 1.7 and 0.17 µg/m$^3$ are associated with excess lifetime risks of leukaemia of $10^{-4}$, $10^{-5}$ and $10^{-6}$, respectively.[6 7]

While it seems unlikely that benzene is a major cause of leukaemia in the general population exposed in the ppb range, children may represent a subpopulation with increased susceptibility.[1 3]

Childhood leukaemias have distinctive features compared with leukaemias in adults. The major subtypes are acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML), accounting for 80% and 15% of cases, respectively, in white populations aged 0–14 years.[8] Both subtypes are thought to develop through a first initiating event in utero (eg, the TEL-AML1 gene fusion whose prevalence in newborns has been estimated at around 1% while it is observed in 25% of ALL cases) followed by further postnatal genetic changes.[8] The 'second hit' might consist of additional idiopathic chromosomal translocations, as well as of exposures to biological, chemical or physical agents.[9] Ionising radiation, benzene, alkylators and topoisomerase II inhibitors are among the few confirmed environmental risk factors for AML, while delayed, dysregulated responses to common infections are quite likely to play a major role in the conversion of preleukaemic clones into overt ALL.[8 9]

Findings from available studies of benzene and childhood leukaemia are inconsistent, possibly due to the use of indirect estimates of exposure and lack of analyses by leukaemia subtype.[10]

To advance current understanding of benzene health effects and susceptibility, studies of paediatric cancers that include estimates of environmental exposure to benzene, rather than surrogate exposure indicators, have been recommended.[11]

Major challenges in pursuing this suggestion include the space variability and time variability of ambient benzene levels, the low exposure levels in children and the inherent susceptibility of case-control studies (the design of choice for aetiological studies of rare disease like childhood cancer) to selection and information bias.

We evaluated the logistic feasibility of an assessment of benzene exposure based on repeated seasonal weekly measurements in breathing zone air samples and outside the children's dwellings, with concurrent determinations of cotinine, *t-t*-muconic acid (MA) and sulfophenylmercapturic acid (S-PMA) in urine, in a pilot investigation within an Italian case-control study on environmental risk factors for childhood leukaemia (SETIL).

Additional objectives of the pilot study were:

- to investigate the relationship between level personal exposure to benzene and putative determinants (atmospheric benzene, second-hand tobacco smoke, individual traits);
- to assess the performance of *t-t*-MA and S-PMA as benzene biomarkers in children;
- to verify the occurrence of participation bias from differential adhesion to the benzene measurement study, and estimate the amount and direction of the distortion;
- to check whether exposures to benzene and to extremely low-frequency magnetic fields (ELF-MF) were correlated, and might eventually exert reciprocal confounding effects on the relationship with childhood leukaemia.

## METHODS

### Study population

Incident cases of childhood leukaemia from 14 Italian regions, aged 0–10 years at diagnosis in 1998–2001, were eligible for enrolment in the SETIL study. Cases were ascertained through the national registry run by the Association of Paediatric Haematology and Oncology. Controls, matched to cases (2 : 1 ratio) on gender, date of birth and region, were randomly selected from population lists. Information on several items concerning the children, their next of kin and dwellings was collected by interviewing parents. All interviewed families were invited to participate in a measurement study of indoor ELF-MF, while subsets of participants were asked to join two side-investigations on exposure to gamma radiation and benzene, respectively.

Eligibility for the benzene pilot study was restricted to 108 childhood leukaemia cases from seven Italian provinces (Turin, Milan, Florence, Rome, Catania,

Palermo and Cagliari), diagnosed between July 2000 and December 2001, and 194 matched controls.

The study protocol was approved by the Piedmont Ethical Committee on 14 January 2002.

### Sampling strategy and devices

Owing to the high daily and seasonal variability of atmospheric benzene concentrations, the protocol called for four repeated seasonal 1-week samplings of breathing zone air per child over 1 year ('personal' air samples), with a concurrent collection of urine samples and atmospheric air samples in proximity of the children's homes ('outdoor' air samples).

Outdoor air sampling would also be performed, with an identical strategy, near the homes of all eligible non-participants.

To study the day-to-day variability in exposure, 24 h repeated personal and indoor samples during four season-specific weeks would be collected from a subset of children and related homes.

Personal air samples were collected by passive samplers (Radiello radial symmetry diffusive sampler) worn by the child during the day and placed at the bedside at night.

Radiello samplers were also used to collect outdoor air samples, placed near the entrance of the dwellings (within 1 m), at a vertical distance from the ground suitable to avoid infringements (2–2.5 m), stored in a plastic case to avoid rain or snow.

At retrieval, the adsorbing cartridges were removed from the diffusive bodies and placed in glass storage tubes. The ID code of the child, along with dates and times of sampling start and end, was recorded on self-adhesive labels stuck on the tubes. The cartridges were sent to a single laboratory (Fondazione Salvatore Maugeri, Padova) for chemical analyses.

Daily urine samples (10 ml, from the last micturition before sleep) were collected for seven subsequent days (70 ml/week) during each seasonal survey. The daily samples were pooled in one plastic vial, and kept in the freezer compartment of the home refrigerator until collection at the end of the week. The vials were transported to the local research centre in cool bags, and stored at –5°C until delivery (packed in dry ice and usually within 2 weeks) to the laboratory (Fondazione Salvatore Maugeri, Pavia).

Field work began between March 2002 and January 2003, and ended in October 2003–July 2004, depending on the local research centre.

### Chemical determinations

Benzene concentrations were determined by an automated thermal desorber (ATD400, Perkin Elmer) coupled to a capillary gas-chromatography system (Autosystem XL, Perkin Elmer). The expanded uncertainty of the method, in the range 2.4–14.3 µg/m$^3$, was shown to be 18%.[12] The limits of detection and quantification, over 1 week exposure, are 0.05 and 0.1 µg/m$^3$.

The urine analyses were performed using a high-pressure liquid chromatography system (Alliance 2690, Waters) equipped with a spectrometric (SM) detector (ZQ, Waters) following a preliminary step of purification of the samples on preactivated solid phase extraction cartridges. The limit of detection (LOD), coefficient of variation (CV) and accuracy of the method were: LOD=1 µg/l, CV%=(1.22)–(1.10), accuracy%=(−2.39)–(3.36) for S-PMA; LOD=20 µg/l, CV%=(1.33)–(1.06), accuracy%=(−2.18)–(3.27) for MA; LOD=1 µg/l, CV %=(1.25)–(1.09), accuracy%=(−2.29)–(3.33) for cotinine.

Further details are provided in Appendix 1.

The chemical determinations were completed by May 2005.

### Statistical analyses

Measurements below the chemical-specific detection limits were assigned half of such values and included in the analyses.

The relationships between personal exposure to benzene and putative determinants (as well as between urinary excretion of benzene metabolites, benzene intake and other covariates) were assessed by generalised least squares (GLS) models for repeated measurements (STATA V.11, xtreg procedure). The GLS model is: $y_{it}=\alpha+X_{it}B+u_{it}+e_{it}$, where i (1 to n) is the number of observations collected at time t (1 to 4) and $u_{it}$ and $e_{it}$ are the error components.

As the concentrations of benzene and urinary analytes were log-normally distributed, we always included log-transformed dependent variables in the models.

We used the OR, calculated from generalised estimating equations (GEE) for repeated individual measurements (STATA V. 11, procedure xtgee), to estimate the association between benzene exposure and dichotomous variables such as case-control or participation status. The general equation of the GEE model is $g\{E(y_j)\}=x_j\beta$, where g is the link function, herein a logit function.

We calculated a participation bias factor following the method suggested by Greenland (bias factor=$(S_{1a}*S_{0b})/(S_{0a}*S_{1b})$), where $S_{1a}$, $S_{0a}$, $S_{1b}$ and $S_{0b}$ denote the probabilities of selection (ie, full participation in the benzene study) for exposed cases, unexposed cases, exposed controls and unexposed controls.[13] When the bias factor equals 1, there is no bias; when it is above or below 1, the true OR will be biased upward or downward, respectively, by the magnitude of this factor.

Multiple regression models were used to analyse the relation between estimated exposures to benzene and ELF-MF.

## RESULTS

### Participation and sampling outcome

Out of 108 cases and 194 controls eligible for inclusion, 46 cases and 60 controls (43% and 31%) agreed to take full part in the benzene side-study (figure 1).

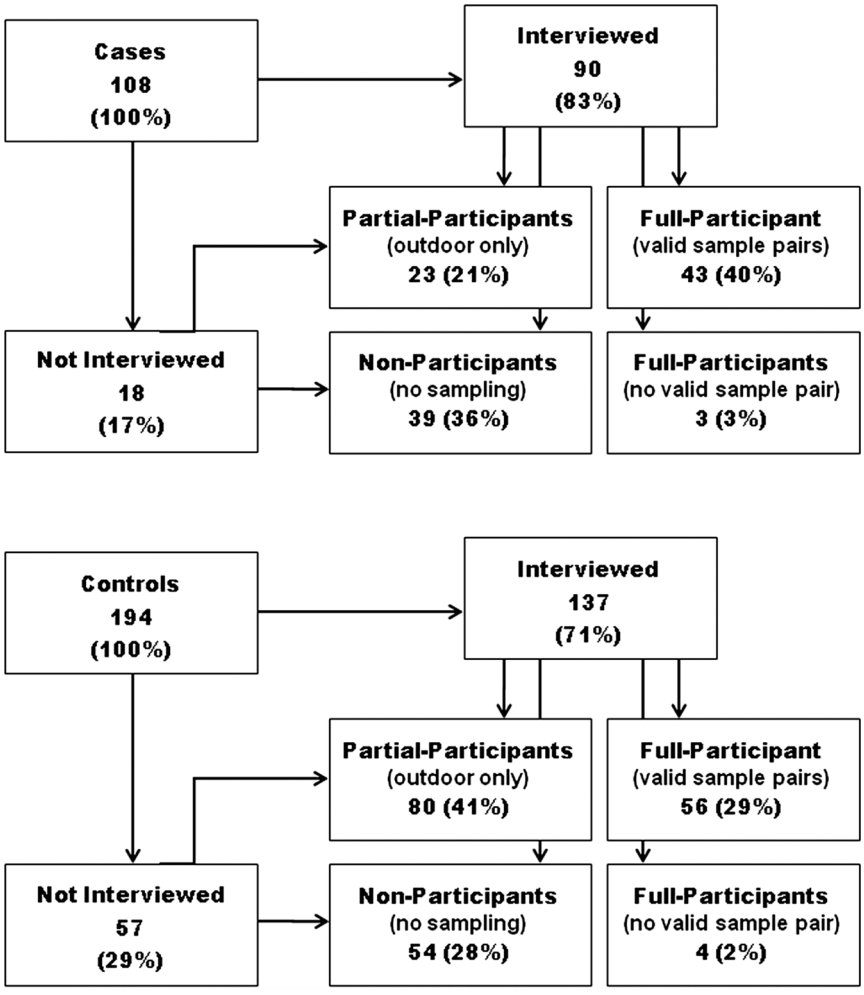


**Figure 1** Children eligible for inclusion and participation rates.

In addition, the parents of 23 cases and 80 controls who refused the personal exposure assessment accepted the outdoor monitoring (partial participation=21% and 41%, respectively).

In all, 1467 air samples were collected. A small percentage (2%) was lost during monitoring (22 samplers stolen, 2 sampler plates broken, 3 cartridges lost), transport (8 missing labels) or chemical analysis (2 cartridges broken on arrival at the laboratory; 1 sample lost due to equipment failure).

Benzene measurements from the day-to-day variability substudy (19% of the total) could not be used because only four control children accepted the 24-h sampling scheme, and were replaced by the calculated weekly averages.

A further 20% of benzene measurements were removed from the data-set due to lack of compliance with the study protocol (indoor samples collected in place of the personal ones from children refusing to wear the sampler; time-or place-mismatch of personal and outdoor samples; 'orphan' personal or outdoor samples; duplicate season-specific measurements; non-participants replaced with children ineligible for the benzene side-study).

For the same reasons, 107 of 417 chemical determinations in urine (26%) were discarded.

Three cases and five controls were excluded from one or more analyses due to lack of complete measurement sets in all seasonal series, and although 89% and 83% of full-participant cases and controls did adhere to all four seasonal surveys, only 37% and 43% of them had four repeated analysable observations.

## Personal characteristics of the children

The families of cases participating in full in the benzene study had been interviewed on average 1.3 years (SD 0.47) after the date of diagnosis, and the control-families 1.5 years (SD 0.46) after the corresponding reference date. The delay between diagnosis and the first series of benzene measurements was 2 years (SD 0.53) for both cases and controls.

Cases and controls were comparable in terms of gender, age and father's attained educational level (table 1). A higher proportion of controls than cases had both parents smoking, and control-mothers were more educated than case-mothers. There were similar proportions of only children in the case and control groups, while firstborn children were more frequent among controls than cases. Early schooling (day-care attendance) was more common in cases than in controls. At the time of the benzene survey, most children

**Table 1** Children included in the pilot study by selected characteristics

| | Cases | | Controls | |
|---|---|---|---|---|
| | N | % | N | % |
| Gender | | | | |
| Female | 25 | 58 | 30 | 54 |
| Male | 18 | 42 | 26 | 46 |
| Age at the survey | | | | |
| (2,4) years | 5 | 12 | 9 | 16 |
| (4,6) years | 21 | 49 | 16 | 29 |
| (6,12) years | 17 | 40 | 31 | 55 |
| Residence* | | | | |
| Turin | 7 | 16 | 9 | 16 |
| Milan | 8 | 19 | 13 | 23 |
| Florence | 3 | 7 | 5 | 9 |
| Rome | 14 | 33 | 15 | 27 |
| Catania | 3 | 7 | 5 | 9 |
| Palermo | 4 | 9 | 6 | 11 |
| Cagliari | 4 | 9 | 3 | 5 |
| Parent smoking† | | | | |
| None | 20 | 47 | 27 | 48 |
| One | 16 | 37 | 18 | 32 |
| Both | 4 | 9 | 11 | 20 |
| Missing | 3 | 7 | 0 | – |
| Father's education† | | | | |
| No qualification | – | – | 1 | 2 |
| Primary school | 17 | 40 | 21 | 38 |
| High school | 17 | 40 | 24 | 43 |
| University degree | 6 | 14 | 10 | 18 |
| Missing | 3 | 7 | – | – |
| Mother's education† | | | | |
| No qualification | – | – | – | – |
| Primary school | 19 | 44 | 17 | 30 |
| High school | 15 | 35 | 26 | 46 |
| University degree | 9 | 21 | 13 | 23 |
| Missing | – | – | – | – |
| Birth order† | | | | |
| Only child | 10 | 23 | 12 | 21 |
| First born | 10 | 23 | 20 | 36 |
| Second born or higher birth order | 23 | 53 | 24 | 43 |
| Age at first schooling† | | | | |
| No schooling yet | 15 | 35 | 16 | 29 |
| <3 years (crèche) | 14 | 33 | 9 | 16 |
| (3,6) years (preschool) | 14 | 33 | 30 | 54 |
| (6–7) years (primary school) | 0 | – | 1 | 2 |
| Home at the time of the benzene survey‡ | | | | |
| Occupied since birth | 28 | 65 | 39 | 70 |
| Moved into after birth and before diagnosis | 13 | 30 | 12 | 21 |
| Moved into after diagnosis and before interview | 1 | 2 | 5 | 9 |
| Moved into after interview | 1 | 2 | – | – |
| Total | 43 | 100 | 56 | 100 |

*At the time of diagnosis or the corresponding reference date for controls.
†Information reported at the interview.
‡The extremely low-frequency magnetic fields measurements, if the parents agreed, were made at the time of the interview.

were still living in the home occupied at birth or in the house they moved into after birth but before the date of diagnosis (cases 95%; controls 91%).

### Level, variability and determinants of personal exposure to benzene

The analyses of level, variability and determinants of personal exposure to benzene were based on 43 cases (39 ALL and 4 AML) and 56 controls, with 261 valid pairs of benzene concentrations in breathing zone and outdoor air (110 from cases and 151 from controls). A large proportion of these children (35%) had a single pair of concurrent measurements, unevenly distributed by season, with a disproportionally high number of summer samples (30 of 35, all but one from a single centre).

The distributions, overall and by season, of benzene concentrations in personal and outdoor air samples, and of cotinine, MA and S-PMA in urine are described in table 2.

Personal exposure to benzene was log-normally distributed (Shapiro-Wilk test=0.938, p<0.001), and the mean benzene level over the individual yearly averages was 3 $\mu g/m^3$ (0.92 ppb).

The distribution of benzene outdoor concentration was skewed to the left in all seasons and the yearly averages were log-normally distributed as well (Shapiro-Wilk test=0.948, p=0.001); the average yearly benzene level near the children's homes was 2.7 $\mu g/m^3$ (0.83 ppb).

Both the outdoor benzene concentrations and personal exposure levels were higher in the cold seasons (autumn-winter) than in the warm ones (spring-summer).

The European limit for benzene in air (5 $\mu g/m^3$) was exceeded by 5% of the yearly average outdoor concentrations, and by 8% of the yearly average levels in breathing zone air samples. A large proportion of autumn and winter measurements were above 5 $\mu g/m^3$ (35% and 25% outdoor; 26% and 30% of the personal exposure estimates).

Cases and controls had similar levels of personal exposure to benzene: the leukaemia OR for a unit increase (1 $\mu g/m^3$) in personal benzene exposure was 0.93 (95% CI 0.77 to 1.13) adjusting for gender, age at the benzene survey (2–4; 4–6; 6–12 years), cotinine in urine (µg/g creatinine), season and province of residence (Turin; Milan; Florence—Rome; Catania—Palermo—Cagliari).

A similar lack of association was found between the odd of disease and benzene concentration outside the children's homes (OR 0.94 (95% CI 0.80 to 1.09)), controlling for gender, age, smoking habits of the parents at the interview (non-smokers, mother or father smoking; both parents smoking), season and province of residence.

Further adjustment for birth order and age at first schooling had no material effect on the observed leukaemia–benzene relationship (personal exposure: OR 0.92 (95% CI 0.75 to 1.13); outdoor benzene: OR 0.95 (95% CI 0.81 to 1.13)).

As cases and controls had comparable levels of benzene exposure, we carried out the analyses illustrated in the forthcoming paragraphs on the whole data-set, although always controlling for caseness.

Urinary cotinine concentration (µg/g of creatinine) was higher in children of smoking parents compared

**Table 2** Benzene concentration in personal and outdoor air samples, and urine level of cotinine and benzene metabolites by season and overall

| | Obs (#) | Mean | SD | G-mean | G-SD | Min | Percentiles p25 | p50 | p75 | Max |
|---|---|---|---|---|---|---|---|---|---|---|
| Benzene in personal air samples (μg/m$^3$) | | | | | | | | | | |
| Spring | 57 | 2.51 | 1.89 | 2.10 | 1.75 | 0.60 | 1.50 | 1.82 | 3.11 | 11.12 |
| Summer | 86 | 2.26 | 1.45 | 1.90 | 1.82 | 0.47 | 1.25 | 1.85 | 3.10 | 8.13 |
| Autumn | 62 | 4.31 | 2.60 | 3.73 | 1.57 | 0.92 | 2.939 | 3.70 | 5.17 | 18.47 |
| Winter | 56 | 4.04 | 1.78 | 3.67 | 1.73 | 1.55 | 2.34 | 4.00 | 5.24 | 9.03 |
| Individual yearly averages | 99 | 3.00 | 1.45 | 2.66 | 1.67 | 0.75 | 2.05 | 2.90 | 3.83 | 9.00 |
| Benzene in outdoor air samples (μg/m$^3$) | | | | | | | | | | |
| Spring | 57 | 2.29 | 1.30 | 1.93 | 1.84 | 0.48 | 1.20 | 1.91 | 3.15 | 5.67 |
| Summer | 86 | 1.94 | 1.20 | 1.65 | 1.75 | 0.39 | 1.12 | 1.58 | 2.28 | 6.92 |
| Autumn | 62 | 3.99 | 2.58 | 3.05 | 1.92 | 0.08 | 1.93 | 3.42 | 5.63 | 11.18 |
| Winter | 56 | 3.80 | 1.86 | 3.25 | 2.35 | 0.15 | 2.40 | 3.66 | 5.20 | 8.31 |
| Individual yearly averages | 99 | 2.70 | 1.41 | 2.33 | 1.78 | 0.27 | 1.59 | 2.37 | 3.63 | 6.92 |
| Cotinine (μg/g creatinine) | | | | | | | | | | |
| Spring | 78 | 3.92 | 7.04 | 1.91 | 3.26 | 0.05 | 1.00 | 1.94 | 3.50 | 49.0 |
| Summer | 78 | 3.20 | 5.52 | 1.50 | 3.59 | 0.09 | 0.82 | 1.68 | 3.71 | 41.4 |
| Autumn | 76 | 4.54 | 8.51 | 1.92 | 3.92 | 0.05 | 1.20 | 1.93 | 4.30 | 48.7 |
| Winter | 74 | 4.36 | 7.38 | 2.32 | 3.01 | 0.10 | 1.20 | 2.30 | 4.80 | 53.5 |
| Individual yearly averages | 98 | 3.73 | 5.99 | 2.14 | 2.67 | 0.30 | 1.08 | 2.09 | 3.58 | 41.9 |
| MA (μg/g creatinine) | | | | | | | | | | |
| Spring | 81 | 104.22 | 69.28 | 87.43 | 1.79 | 17.00 | 60.27 | 82.00 | 126.99 | 349.00 |
| Summer | 79 | 140.40 | 226.73 | 92.30 | 2.16 | 13.33 | 56.54 | 83.00 | 131.76 | 1680.00 |
| Autumn | 76 | 128.24 | 124.04 | 99.57 | 1.94 | 30.21 | 60.16 | 102.48 | 147.21 | 893.04 |
| Winter | 74 | 119.09 | 100.15 | 95.30 | 1.86 | 26.00 | 65.00 | 86.00 | 129.00 | 591.00 |
| Individual yearly averages | 98 | 116.65 | 84.89 | 101.06 | 1.62 | 46.42 | 73.33 | 92.66 | 122.50 | 593.42 |
| S-PMA (μg/g creatinine) | | | | | | | | | | |
| Spring | 81 | 1.13 | 0.60 | 1.00 | 1.62 | 0.21 | 0.80 | 1.00 | 1.30 | 3.70 |
| Summer | 79 | 1.12 | 0.54 | 1.02 | 1.54 | 0.41 | 0.72 | 1.00 | 1.39 | 3.30 |
| Autumn | 76 | 1.53 | 0.93 | 1.33 | 1.67 | 0.49 | 0.97 | 1.29 | 1.84 | 5.80 |
| Winter | 74 | 1.37 | 0.60 | 1.23 | 1.64 | 0.15 | 1.00 | 1.20 | 1.60 | 3.40 |
| Individual yearly averages | 98 | 1.28 | 0.50 | 1.20 | 1.43 | 0.56 | 0.94 | 1.20 | 1.46 | 2.97 |

MA, muconic acid; S-PMA, sulpho-phenylmercapturic acid.

with children of non-smokers, and children with both parents smoking excreted a larger amount of cotinine than children with one parent smoking (Appendix table A). Cotinine levels were higher in winter than in other seasons, and higher in children from central and southern Italy (Florence, Rome, Palermo, Catania and Cagliari) than in children from northern provinces (Turin and Milan). The high between-subject versus within-subject $R^2$ ratio (0.51/0.07) is worth noting.

Personal benzene exposure was strongly influenced by outdoor benzene concentrations (table 3A), and apparently not affected by gender or age; the season showed a modifying effect, with increasing levels of personal exposure during autumn and winter; the fraction of variability explained by the model was higher for the within-subject component than for the between-subject one.

Exposure to second-hand tobacco smoke (estimated by cotinine excretion or by parental smoking habits) showed a trivial influence on personal exposure to benzene. The inclusion of urinary cotinine (μg/g creatinine) in the model described in table 3A slightly decreased its goodness of fit ($R^2$ overall=0.46; Wald $\chi^2$=189.49; $R^2$ within=0.55; $R^2$ between=0.35; β (cotinine) =0.012; 95% CI=−0.003 to 0.03)); an alternative model, including smoking habits of the parents, did not perform any better ($R^2$ overall=0.46; Wald $\chi^2$=216.44; $R^2$ within=0.52; $R^2$ between=0.39; β (one parent smoking) =0.14; 95% CI=−0.02 to 0.31; β (both parents smoking) =0.17; 95% CI=−0.06 to 0.39).

Children from central Italy (Florence and Rome) tended to have lower benzene concentrations in breathing zone air samples compared with residents in other provinces, all other things being equal (table 3A), possibly because of residual confounding from the lack of samples collected in Rome other than in summer. We tried to verify this hypothesis by restricting the analyses to children with at least two series of measurements in different seasonal periods (cold and warm). The data-set reduced to 61 subjects (25 cases and 36 controls) and 220 pairs of personal-outdoor benzene measurements. Actually, children from Florence still showed (not significantly) lower levels of personal exposure to benzene (β=−0.27; 95% CI=−0.56 to 0.03; p=0.074)

**Table 3** Personal exposure to benzene (ln μg/m$^3$) by outdoor benzene concentration, cotinine, gender, age, season, province of residence and caseness

| (A) Whole data-set (261 observation, 99 children) | β | 95% CI (β) | p(Z) |
|---|---|---|---|
| Outdoor benzene(μg/m$^3$) | 0.151 | 0.12 to 0.19 | <0.001 |
| Gender (male vs female) | −0.052 | −0.21 to 0.11 | 0.522 |
| Age (at the benzene survey) | Reference 6–12 years | | |
| (2–4) years | 0.027 | −0.20 to 0.25 | 0.814 |
| (4–6) years | −0.147 | −0.32 to 0.03 | 0.098 |
| Season | Reference Spring | | |
| Summer | −0.027 | −0.18 to 0.12 | 0.717 |
| Autumn | 0.317 | 0.16 to 0.48 | <0.001 |
| Winter | 0.330 | 0.17 to 0.49 | <0.001 |
| Residence | Reference Turin | | |
| Milan | −0.038 | −0.28 to 0.20 | 0.759 |
| Florence—Rome | −0.208 | −0.45 to 0.03 | 0.091 |
| Catania—Palermo—Cagliari | −0.086 | −0.31 to 0.13 | 0.443 |
| Case vs control | −0.039 | −0.19 to 0.12 | 0.623 |
| $R^2$ overall=0.4617 (within=0.5364; between=0.3603); Wald $\chi^2$=234.0; p<0.0001 | | | |

| (B) Restricted data-set (≥2 repeats; 175 observations, 61 children) | β | SE (β) | p(Z) |
|---|---|---|---|
| Outdoor benzene(μg/m$^3$) | 0.123 | 0.020 | <0.001 |
| Cotinine (μg/g creatinine) | 0.023 | 0.011 | 0.039 |
| Gender (male vs female) | −0.057 | 0.116 | 0.623 |
| Age (at the benzene survey) | Reference 6–12 years | | |
| (2–4) years | 0.050 | 0.161 | 0.757 |
| (4–6) years | −0.199 | 0.121 | 0.100 |
| Season | Reference Spring | | |
| Summer | −0.055 | 0.081 | 0.494 |
| Autumn | 0.382 | 0.087 | <0.001 |
| Winter | 0.351 | 0.086 | <0.001 |
| Residence | Reference Turin | | |
| Milan | 0.038 | 0.155 | 0.807 |
| Florence | −0.323 | 0.195 | 0.099 |
| Catania—Palermo—Cagliari | −0.00001 | 0.138 | 1.000 |
| Case vs control | −0.073 | 0.107 | 0.498 |
| $R^2$ overall=0.4858 (within=0.5564; between=0.3544); Wald $\chi^2$=171.89; p<0.0001 | | | |

compared with children from Turin (data not shown). In the restricted data-set, however, independent effects of both outdoor benzene and urinary cotinine levels on personal benzene exposure were observed (table 3B).

### Benzene intake and urinary excretion of benzene metabolites

Ninety-eight children (43 cases and 55 controls) and 310 pairs of urine and breathing zone air measurements (138 from cases and 172 from controls) were available for the analyses of the urinary excretion of benzene metabolites (MA and S-PMA) in relation to personal exposure to benzene.

Urinary concentrations of S-PMA (ln μg/g creatinine) were related to personal exposure to benzene (table 4, Model 1). Youngest children (2–4 years at the benzene survey) excreted higher level of S-PMA compared with children aged 6–12 years, all other conditions being equal, and urinary concentrations of S-PMA were higher in samples collected during the cold seasons compared with spring samples. The model, however, explained just 19% of the overall S-PMA variability. In an alternative model, including outdoor benzene concentrations and urinary cotinine in place of personal benzene exposure, we also observed an effect of the nicotine biomarker on S-PMA excretion (table 4, Model 2).

On the contrary, neither benzene concentrations in breathing zone air samples nor outdoor benzene concentrations or cotinine levels explained the intraindividual and interindividual variability in urinary levels of MA, controlling for gender, age, season, area of residence and caseness (data not shown).

**Table 4** Urinary excretion of S-PMA (ln μg/g creatinine) by personal benzene exposure (model 1) or outdoor benzene concentration plus urinary cotinine (model 2), controlling for gender, age, season, province of residence and caseness

| Model 1 (310 observations, 98 children) | β | 95% CI (β) | p(Z) |
|---|---|---|---|
| Personal benzene exposure (μg/m$^3$) | 0.031 | 0.004 to 0.06 | 0.024 |
| Gender (male vs female) | −0.027 | −0.16 to 0.11 | 0.695 |
| Age (at the benzene survey) | Reference 6–12 years | | |
| (2–4) years | 0.395 | 0.22 to 0.57 | <0.001 |
| (4–6) years | −0.011 | −0.16 to 0.14 | 0.890 |
| Season | Reference Spring | | |
| Summer | 0.043 | −0.09 to 0.17 | 0.514 |
| Autumn | 0.250 | 0.11 to 0.38 | <0.001 |
| Winter | 0.156 | 0.01 to 0.30 | 0.033 |
| Residence | Reference Turin | | |
| Milan | 0.007 | −0.21 to 0.23 | 0.949 |
| Florence—Rome | 0.013 | −0.18 to 0.21 | 0.898 |
| Catania—Palermo—Cagliari | 0.068 | −0.14 to 0.27 | 0.514 |
| Case versus control | 0.053 | 0.647 | 0.415 |
| $R^2$ overall=0.1894 (within=0.1263; between=0.2174); Wald $\chi^2$=58.97; p<0.0001 | | | |
| **Model 2 (214 observations, 98 children)** | | | |
| Outdoor benzene concentration (μg/m$^3$) | 0.009 | −0.02 to 0.04 | 0.605 |
| Cotinine (μg/g creatinine) | 0.014 | 0.001 to 0.03 | 0.040 |
| Gender (male vs female) | −0.012 | −0.16 to 0.14 | 0.875 |
| Age (at the benzene survey) | Reference 6–12 years | | |
| (2–4) years | 0.308 | 0.08 to 0.54 | 0.008 |
| (4–6) years | 0.055 | −0.11 to 0.22 | 0.516 |
| Season | Reference Spring | | |
| Summer | −0.040 | −0.18 to 0.10 | 0.582 |
| Autumn | 0.200 | 0.04 to 0.36 | 0.012 |
| Winter | 0.082 | −0.07 to 0.24 | 0.305 |
| Residence | Reference Turin | | |
| Milan | −0.053 | −0.28 to 0.18 | 0.657 |
| Florence—Rome | 0.048 | −0.18 to 0.28 | 0.687 |
| Catania—Palermo—Cagliari | 0.003 | −0.21 to 0.22 | 0.974 |
| Case vs control | 0.011 | −0.14 to 0.16 | 0.882 |
| $R^2$ overall=0.1158 (within=0.1423; between=0.0925); Wald $\chi^2$=27.59; p=0.0063 | | | |

S-PMA, sulfo-phenylmercapturic acid.

### Bias due to differential participation

The analysis of participation bias included 66 cases (43 full-participant and 23 partial-participant) and 136 controls (56 and 80), with 652 measurements of outdoor benzene concentrations (135 and 175 from full-participant cases and controls; 81 and 261 from partial-participant cases and controls).

Benzene concentrations near the homes of full-participant controls were significantly lower than those in proximity of partial-participants' dwellings (OR=0.88; 95% CI 0.80 to 0.97), adjusting for gender, age, season and place of residence, while there was no difference in ambient benzene levels between participant and non-participant cases (OR=0.95; 95% CI 0.82 to 1.09). As participation in the study was also associated with the case-control status, assuming a causal association between exposure and disease, a selection bias might ensue. However, as parents of more exposed controls were less willing to accept to be interviewed, an upward distortion would be expected, which is at odds with the apparent lack of association between personal benzene exposure and leukaemia risk in the current study.

To the aim of the current analysis, personal exposure to benzene was dichotomised around the median (3.25 μg/m$^3$), the 75th percentile (4.34 μg/m$^3$) or 5 μg/m$^3$ (the current limit for airborne benzene in Europe). The amount and direction of bias were found to depend on the cut-point chosen (Appendix table B), whereas no bias is expected when the exposure is categorised around the median (bias factor=1.03), and biases in the opposite directions are predicted using cut-offs at p75 and at 5 μg/m$^3$ (0.64 and 1.42, respectively).

### Relationship between exposures to benzene and ELF-MF

Children with benzene and ELF-MF measurements made at the same house qualified for inclusion in the analysis of the relationship between estimated exposures to these agents. As only 35 cases and 46 controls met such criteria when benzene concentrations in breathing zone air samples were used as the exposure indicator, we performed the analysis on 48 cases and 77 controls with place-comparable pairs of average yearly outdoor benzene concentration ($\mu g/m^3$) and 48 h TWAs of ELF-MF level in the child's bedroom (ln μT).

There was a positive association between estimated exposures to ELF-MF (dependent variable) and benzene (β=0.177; 95% CI 0.06 to 0.29; p=0.002); the multivariable regression model (including gender, age, province of residence, caseness and participation in the benzene pilot study as covariates) explained 16% of the variability in the dependent variable (F (10, 114 df)=2.13; p>F=0.0271). A steeper increase in the ELF-MF level per unit increase in outdoor benzene concentration (β=0.520; 95% CI 0.09 to 0.95; p=0.019) was seen among the 81 children fully participating in the benzene pilot-study compared with the 44 partial-participants (Appendix table C).

Similar results, with a more accentuated increase in the indoor magnetic induction level per unit increase in outdoor benzene concentration (β=0.272; 95% CI=0.09 to 0.45; p(t)=0.003; $R^2$=0.19), were observed in the restricted data-set of 86 children with ≥2 weekly samplings in alternate seasons.

## DISCUSSION

We have carried out a pilot case-control study of childhood leukaemia and exposure to benzene assessed by repeated individual measurements made on average 2 years after diagnosis. The pilot study included side-investigations aimed at evaluating the performance of two biological indicators of benzene exposure in children, at estimating amount and direction of a possible participation bias and at assessing the relation between estimated exposures to benzene and ELF magnetic fields.

Owing to the relatively low incidence of childhood cancers (10–15 for 100 000 person-years in the 0–14 year range in most industrialised countries), the case-control approach is the design of choice for analytical epidemiological studies about potential risk factors for these diseases. Such a study design, however, is inherently prone to measurement errors stemming from the retrospective reconstruction of the exposures of interest, and to differential participation leading to control samples not being representative of the study base. Therefore, findings from observational epidemiological studies of postulated determinants for childhood malignancies are often inconsistent and always require a cautious and thoughtful interpretation.[14]

Although based on small numbers, some of the findings from the current study have a certain factual and methodological interest.

Repeated samplings of breathing and outdoor air are indeed needed to account for the seasonal variability in environmental benzene levels.[15 16]

On average, children participating in the current study appear to experience mean yearly levels of personal exposure to benzene not exceeding the European guidelines (although 8% of the yearly mean levels were above 5 $\mu g/m^3$).

What we a priori considered to be the main sources of benzene exposure for children (ambient benzene levels and second-hand tobacco smoke) explained no more than half of the overall variability in personal exposure, which indicates the need to identify other sources of exposure particularly relevant, perhaps, during the cold seasons. In fact, in autumn-winter compared with spring-summer, we observed higher levels of personal exposure to benzene, of urinary cotinine and of S-PMA excretion, all other things being equal. These findings might be due to the lower ventilation rates in homes and schools during the cold seasons, to winter-specific sources of indoor benzene concentrations not considered in the current survey (eg, fireplaces or other combustion sources), and/or to the seasonal variability in daily patterns of time spent in different microenvironments (eg, within cars or buses).[17]

Some case-control studies have suggested an association between exposure to traffic density and childhood leukaemia[18–21]; however, negative findings have also been reported.[22–25] Positive associations between the incidence of ALL in children and residential proximity to petrol stations were observed in three case-control studies.[23 26 27] An increased risk of childhood leukaemia in relation to estimated exposure to benzene was observed in a small Italian study,[28] but not in a much larger case-control study carried out in Denmark and based on a sophisticated and validated exposure modelling.[29]

To our knowledge, there is no previous study of childhood leukaemia and measured personal benzene exposure. Moreover, as only children aged 0–10 years at diagnosis were eligible for the SETIL study, the large majority of cases included in the current investigation were pre-B ALL.

Cases and controls did not differ in terms of exposure to benzene, estimated either by benzene level in personal air samples or through outdoor benzene concentration, but the interpretation of this finding is hampered by the retrospective exposure assessment and the low statistical power of this preliminary investigation. That notwithstanding, owing to the design being based on repeated individual observations, the risk estimates have quite narrow CIs. Thus, the findings from this pilot study, in accordance with the limited evidence for an association between exposure to benzene and ALL,[3 5] might also suggest that the levels of benzene exposure experienced by children living in Italian towns do not entail a detectable increase in the risk of ALL.

Current perspectives on the causes of childhood ALL increasingly point toward an aetiological role of altered patterns of infections and related immune stimulation

during the first years of life, and one piece of supporting evidence is the consistent observation of an inverse association between ALL risk and day-care attendance.[30] Studies of childhood ALL and birth order, on the other hand, have provided inconsistent result.[31] Neither age at first schooling nor birth order confounded the relation between childhood leukaemia and indicators of benzene exposure in the current study.

S-PMA concentration measured in repeated weekly samples of the last micturition before sleep was found to reflect personal exposure to benzene, although the available covariates explained a small fraction of the within-subject and between-subject variability of this benzene metabolite. This is a quite surprising result, considering that S-PMA is believed to represent less than 1% of urinary benzene metabolites for exposures to benzene at air concentrations between 0.1 and 10 ppm.[32]

Benzene exposure proved to be unable to explain the variability of MA urinary excretion observed in our children, consistent with findings from a previous Italian study.[33] The low statistical power of the study, the low level of benzene exposure and the lack of adjustment for the confounding effect of dietary intake of sorbic acid (a common food additive) may explain this finding.[34]

Full-participation rates were low, in line with a general tendency to decreasing participation rates, especially in epidemiological studies requiring adherence to complex measurement protocols.[14 35] That notwithstanding, the outdoor monitoring was accepted by a fairly satisfactory proportion of families (64% and 72% of eligible cases and controls). This is an encouraging result, given the strong correlation between personal benzene exposure and ambient benzene level observed in the current study.

We observed a differential participation bias, which underscores the need to plan parallel bias analyses in any case-control study.[36] The dependence of the participation bias factor on the cut-point chosen to dichotomise the exposure variable is of methodological interest.

The positive association between the 48 h TWA of ELF-MF induction in the child's bedroom and the average yearly concentrations of outdoor benzene will need to be considered in the interpretation of findings from the analyses of childhood leukaemia risk in relation to 50 Hz MF in the SETIL case-control study.

Incidental failures during sample collection, transport or chemical analysis accounted for a negligible proportion of lost air or urine samples. However, substantial percentages of chemical measurements could not be included in the current analyses because of a misunderstanding of the sampling protocol.

The day-to-day variability substudy was clearly too demanding to be acceptable.

In conclusion, the current pilot study suggests that epidemiological studies of childhood leukaemia risk and measurement-based estimates of exposure to benzene are challenging but logistically feasible (provided that the study protocol specifies every single sampling detail and nothing is considered so obvious as to be omitted). Such an exposure assessment method could be considered by epidemiologists willing to involve in the 'genome–exposome' approach to gain further insight into the relationship between benzene exposure and childhood leukaemia risk, with priority given to AML.[2 37–39] Owing to the low incidence rates of AML in children, however, international multicentre studies are needed to address this topic.

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**Contributors** SL designed the study, planned the statistical analyses and drafted the manuscript. DF carried out the statistical analyses. AR was in charge of the data management, quality control and descriptive statistical analyses. PS and SN collaborated on the study design, and were responsible for the chemical analyses. RR, as manager of the AIEOP childhood leukaemia registry, performed the case ascertainment. SC, VT, PC, FF, LM, LB and CM were the principal investigators of the local centres collaborating on the benzene pilot study in the framework of the SETIL multicentre case-control study. All the authors critically revised the early drafts, collaborated on the discussion of the study findings and approved the final version of the manuscript.

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