

Leukemia After Exposure to Benzene: Temporal Trends and Implications for Standards

Murray M. Finkelstein, PhD, MDCM^{1,2*}

Background Benzene is a human leukemogen. Risk assessment, and the setting of occupational and environmental standards, has assumed that risk is constant in time after a unit of exposure. Leukemia risk is known to vary with time after exposure to ionizing radiation.

Methods A matched case-control study of leukemia risk in relation to the temporal pattern of benzene exposures was performed using data from the National Institute of Occupational Safety and Health.

Results Leukemia risk following exposure to benzene varied with time in a manner similar to that following exposure to ionizing radiation. More recent exposures were more strongly associated with risk than were more distant ones. There was no significant relation between leukemia death and benzene exposures incurred more than 20 years previously.

Conclusions Recent analyses of specific occupational and environmental carcinogens, including benzene and radon, have indicated that cancer risk tends to decline as the time from exposure increases. This suggests that standards for the control of occupational or public risk must be selected to control exposures over a narrower time frame than the usual lifetime one. In the case of benzene, it would appear that risk is attributable primarily to exposures incurred during the previous 10 to 20 years, with exposures in the most recent 10 years being the most potent. To limit risk, exposures must be controlled during that interval. It is important that epidemiologists explore the temporal pattern of risk in their studies to facilitate the risk assessment of other carcinogens. *Am. J. Ind. Med.* 38:1–7, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: benzene; leukemia; risk assessment; carcinogenesis

INTRODUCTION

Knowledge of the temporal evolution of risk after exposure to a carcinogen is important both for the setting

of standards to protect workers and the public, and, for the understanding of the mechanisms of carcinogenesis. Epidemiologists frequently exclude from their calculations those exposures received in the 5 or 10 years prior to death on the grounds that these exposures are too close to the outcome to be relevant to causation. It is not widely recognized, however, that exposures received in the distant past might not retain much “potency” with respect to cancer incidence and it is rare to find epidemiologists discounting remote exposures in their estimates of “cumulative exposure.”

There is epidemiologic evidence that risk after exposure to a carcinogen varies with time. Survivors of

¹Family Medicine Centre, Mt Sinai Hospital, Toronto

²Program in Occupational Health and Environmental Medicine, McMaster University, Hamilton, Ontario

*Correspondence to: Dr. Murray Finkelstein, Family Medicine Centre, Suite 413, Mt Sinai Hospital, Toronto, Ontario, Canada, M5G 1X5. E-mail: murray.finkelstein@utoronto.ca

the atomic bombings in Japan experienced an increased risk of leukemia that peaked within 10 years after exposure and which had largely disappeared by 20 years after exposure [Darby et al., 1985]. The relative risk for breast cancer increases until 15 years after exposure to low-LET radiation and then begins to decrease [NAS/NRC, 1990]. The risk of lung cancer decreased by a factor of 5 over the period of 10 to 30 years postexposure to low-LET radiation [NAS/NRC, 1990]. An analysis of 11 uranium miner cohorts found that excess relative risk increased for 5–10 years after exposure to radon and thereafter slowly decreased [Lubin et al., 1994]. In contrast to those findings, there was no evidence of temporal change in the atomic bomb survivors' risk of death from digestive cancers [NAS/NRC, 1990].

The solvent, benzene, is a human carcinogen [IARC, 1982]. In 1987, Rinsky and colleagues [Rinsky et al., 1987] published the results of an epidemiologic risk assessment for benzene. Their study was based on the experience of workers at three plants that manufactured a rubber film using benzene as a solvent. The standardized mortality ratio (SMR) for leukemia was 337 (95% CI: 154–641). With stratification according to the levels of cumulative exposure, the SMRs for leukemia increased from 109 to 322, 1186, and 6637 with increases in cumulative benzene exposure from less than 40 parts per million-years (ppm-years) to 40 to 199, 200 to 399, and 400 or more ppm-years, respectively. Rinsky used “cumulative exposure” as the exposure metric; that is, all increments of exposure were summed over the occupational history and each increment was given equal weighting, no matter when it occurred. I obtained a copy of this data file from Robert Rinsky of the National Institute for Occupational Safety and Health (NIOSH) with the intention of re-analyzing it to explore any time dependencies in the risk of leukemia following exposure to benzene. I found that there is evidence to suggest that leukemia risk does vary with time after exposure, and that this time dependency is similar to that observed among individuals exposed to ionizing radiation. This suggests that time dependency must be taken into account in order to set adequately protective exposure standards for workers and the public.

Background

The background to the original study was described by Rinsky and colleagues [Rinsky et al., 1987]. Briefly, the study was based on the experience of workers at three plants that manufactured a rubber film. Natural rubber was dissolved in benzene and spread on a conveyor. The benzene was then evaporated and recovered, and the resultant thin film was stripped from the conveyor, rolled, and milled. Rubber hydrochloride was manufactured at Location 1 from 1939 until April 1976. Production at Location 2 was carried out in two separate plants. At the first, it began as a research and development project; commercial production then

began in 1936 or 1937 and continued until 1949, when the second plant began operation. This operation continued until 1965. Operations at all three plants were essentially identical. Industrial hygiene records describing past atmospheric concentrations of benzene at the plants were available from government and company records. Gaps in the data were filled by estimating exposures.

All nonsalaried white men employed in a rubber hydrochloride department for at least 1 day between January 1, 1940 and December 31, 1965, were eligible for the study. Vital status was ascertained for the cohort through December 31, 1981. Death certificates for all the known deaths were obtained and coded by a qualified nosologist according to the rules of the International Classification of Diseases that were in effect at the time of death. Cohort members who were not traced were considered to be alive at the study's ending date. Since the Rinsky study, NIOSH has extended its follow-up of this cohort for an additional 6 years through the end of 1987, now including women in the follow-up. I was provided with the updated data file which identified the deaths of 15 workers (14 men, 1 woman) with leukemia.

METHODS

The goal of the analysis was to identify any temporal variation of leukemia risk following exposure to benzene. This was accomplished by conducting a case-control study in which the exposures of subjects with leukemia, and matched controls, were compared at various times before the death of the case. If the exposures of cases and controls differed during some period prior to death it might be inferred that exposures during that time were causally related to mortality. Conversely, if exposures of cases and controls were similar during a preceding time period, it might be concluded that those exposures were unrelated to the risk of death.

Each of the 15 subjects with leukemia was matched with control subjects who were born within 3 years of the case subject, who had been hired before, and were alive at, the date of death of the case. Subjects were allowed to serve as controls for more than one case. The matching ratio was 6:1 for the female case subject, 24:1 and 27:1 for two other subjects, and exceeded 50:1 (maximum 333:1) in all the other strata.

The NIOSH data file contained a job code, and the start and finish dates, for each job performed by each subject. A computer program was written to compute annual benzene exposures (parts per million in air x fraction of year in exposure; ppm-yrs) for each subject by combining the job information with the record of estimated annual benzene exposures for each job. To permit exploration of temporal variability, benzene exposures were computed for members of each matched set for time windows comprising 1 to 4, 5 to

9, 10 to 14, 15 to 19, 20 to 24, and 25 to 29 years before the death of the case subject in that set. In addition to computing the exposures in the individual time windows, a Benzene Exposure Index (BEI) was computed using Principal Components methodology [Sharma, 1996] which accounted for correlations among the various time windows and provided weights for the exposures in each window.

The relations between leukemia risk and various measures of benzene exposure were investigated with Conditional Logistic Regression. Exposure measures included cumulative exposure (sum of annual exposures from 29 years to 1 year before the death of the case subject), exposures in each of the individual time windows, and the exposures as indexed by the first 2 Principal Components. Principal Components Analysis and Conditional Logistic Regression were performed with SPSS [SPSS Inc., 1999]. Statistical variability was assessed with bootstrap resampling [Efron and Tibshirani, 1994] using Splus [Mathsoft, 1999]. The fits of regression models were compared with the Akaike Information Criterion ($AIC = -2 \times \log \text{likelihood} + 2 \times \text{degrees of freedom}$). Models which included polynomial terms in exposure were considered in addition to models containing solely a first order exposure term. The model with the lowest AIC was considered the best fitting model [Long, 1997]. The fits of the models with continuous exposure variables were explored visually by comparison with the fit of categorical models in which exposure was divided among three categories.

RESULTS

Conditional Logistic Regression Analyses of Leukemia Risk

Table I shows the results of the regression analyses using various measures of benzene exposure.

Cumulative exposure

Leukemia risk was significantly associated with cumulative exposure (Likelihood Ratio 5.07; $P = 0.024$). The confidence interval for the coefficient, $\beta = 0.0028$, overlapped with that for the coefficient reported by Rinsky et al. [1987] ($\beta = 0.0126, 0.0028-0.0224$) who analyzed the nine cases of leukemia in the cohort at the time.

Exposures in time windows before the death of the case subject

The regression coefficients for the individual exposure windows decreased monotonically from the maximum for the window 1 to 4 years before death. The coefficients for those windows more than 14 years before the death of the case were not statistically significant; that is, there were no significant differences between the exposures of the case subjects and their controls more than 14 years before the death of the case.

TABLE I. Regression Coefficients for Leukemia Risk in Relation to Various Measures of Benzene Exposure. The Odds Ratio is Obtained by Exponentiating the Coefficient

Measure of benzene exposure	Coefficient (95% C.I.)	P-value	AIC ^a
Cumulative exposure (ppm-yrs)	0.0028 (0.0008–0.0048)	0.0052	131.1
Individual exposure windows			
1 to 4 years before death (E_{1-4})	0.0215 (0.008–0.035)	0.0012	
5 to 9 years before death (E_{5-9})	0.0187 (0.009–0.029)	0.0003	
10 to 14 years before death (E_{10-14})	0.0155 (0.006–0.025)	0.0012	
15 to 19 years before death (E_{15-19})	0.0062 (–0.004–0.017)	0.24	
20 to 24 years before death (E_{20-24})	0.0059 (–0.008–0.020)	0.41	
25 to 29 years before death (E_{25-29})	–0.0029 (–0.0040–0.027)	0.84	
Principal components model			125.2
Principal component 1	0.19 (–0.17–0.54)		
Principal component 2	0.47 (0.13–0.81)		
Benzene exposure index			
BEI	0.95 (0.47–1.43)	0.0001	123.5
Square of benzene exposure index			
BEI-Squared	0.24 (0.13–0.36)	<0.0001	122.1

^aThe Akaike Information Criterion (AIC) allows comparison of the fits of competing statistical models to the data. The model with the lowest AIC is considered the best fitting model.

In order to account for the entire exposure history, all the exposure windows were entered into a logistic regression model. Although the overall fit was significant ($P = 0.015$), the individual coefficients were poorly estimated and were not significant because of correlations among the exposure windows.

Principal components analysis

To address multicollinearity, an exposure index was computed using the method of Principal Components. Principal components were computed using the correlation matrix so as not to more heavily weight those time windows with the greatest variances. The first two principal components, accounting for 74% of the variance, had eigenvalues greater than 1. The other four, with eigenvalues from 0.16 to 0.68, were not retained for further analysis. Table II shows the weights computed for the contribution of each time window to the composition of the two retained principal components. The first principal component can be interpreted as a weighted average of the exposures in each of the time windows, while the second is the difference between more recent and more remote exposures. Bootstrap resampling found that the differences between the observed weights and the mean weights from 1000 replications were 1% or less and that the coefficients of variation were 36% or less.

A conditional logistic regression model was fitted using the first two principal components as the exposure variables. In keeping with the general principles, both components were forced into the model [Glantz and Slinker, 1990]. The AIC for this model, 125.2, indicated a more parsimonious fit than that for the model containing the six individual exposure windows (AIC = 130.5). This model also provided a better fit to the data than did the simple cumulative exposure model (AIC = 131.1).

From the logistic regression model in which exposure was represented by the first two principal components,

TABLE II. Results of the Principal Components Analysis of the Exposure Windows

Exposure window	Coefficient of PC 1 ^a	Coefficient of PC 2
1 to 4 years before death	0.09	0.439
5 to 9 years before death	0.139	0.451
10 to 14 years before death	0.256	0.229
15 to 19 years before death	0.324	-0.067
20 to 24 years before death	0.316	-0.222
25 to 29 years before death	0.286	-0.241

^aPC1 = Principal Component 1 = $0.09 \times E_{1-4} + 0.139 \times E_{5-9} + 0.256 \times E_{10-14} + 0.324 \times E_{15-19} + 0.316 \times E_{20-24} + 0.286 \times E_{25-29}$, and similarly for Principal Component 2. The time window variables have been standardized for this analysis.

the odds ratio for leukemia in relation to benzene exposure is:

$$\text{Odds Ratio} = \exp(\beta_1 \times \text{PC}_1 + \beta_2 \times \text{PC}_2).$$

Re-expressing this equation in terms of the original exposure windows variables (using the coefficients of Table II and accounting for the standardization of the variables) the result is

$$\begin{aligned} \text{Odds Ratio} = & \exp(0.011 \times E_{1-4} + 0.0098 \times E_{5-9} \\ & + 0.0067 \times E_{10-14} + 0.0012 \times E_{15-19} \\ & - 0.0019 \times E_{20-24} - 0.005 \times E_{25-29}) \end{aligned}$$

where the E-terms represent exposures in the various time windows.

There are several important features to this equation. In contrast to the cumulative exposure model, in which a unit of exposure during all time intervals is given a uniform weighting ($\beta = 0.0028$), the exposures in the various time windows in this model are each weighted differently. A unit of exposure in the time window closest to the death of the case receives the greatest weighting, with the weight decreasing monotonically for the more distant exposures. The coefficients for the exposures 20 or more years prior to death are small and negative. Although it is plausible that remote exposures might be protective against the development of leukemia, these coefficients probably represent only noise. In the analyses that follow, I have set them equal to 0 in the creation of the BEI.

The BEI thus includes weighted contributions for exposures during the previous 20 years, and is:

$$\begin{aligned} \text{BEI} = & 0.011 \times E_{1-4} + 0.0098 \times E_{5-9} + 0.0067 \times E_{10-14} \\ & + 0.0012 \times E_{15-19}. \end{aligned}$$

I fitted Conditional Logistic Regression Models using the BEI, and BEI-squared, as measures of exposure. As shown in Table I both measures of exposure provided highly significant fits to the data, with the square of the BEI providing a slightly better fit. (The AIC for a linear-quadratic model was 124.0.) Both of these models provided a better fit than did cumulative exposure (AIC = 131.1) and the model with six time windows (AIC = 130.5). Examination of residuals and dfbetas showed no serious problems with the BEI models.

DISCUSSION

Benzene is a cause of human leukemia. The analysis presented here has demonstrated that the increased relative

risk of leukemia following exposure to benzene varies with time. The methodology was somewhat unusual, in that, rather than looking forward from the date of exposure to observe the evolution of risk, I looked backward from the date of death of the case subjects and compared the exposures of case and control subjects in time windows prior to the death of the cases. As is usual in case-control studies one infers that, in the absence of differences in exposure between cases and their matched controls, exposure was not related to increased risk of disease.

As shown in Table I, there was no significant difference in the benzene exposures of subjects with leukemia and their matched controls 15 or more years prior to the death of the case subject. The greatest risk of leukemia was related to exposures incurred in the previous 10 years. Many subjects were employed for extended periods and experienced chronic exposure to benzene. Exposures in each time window thus tended to be correlated with those in neighboring windows. I used the risk pattern in the cohort to derive a BEI, a weighted average of exposures in time windows prior to the death of the case subject. Assigning exposures in the interval 1–4 years prior to the death of the case a weight of 1.0, the relative weights in the intervals 5–9, 10–14 and 15–19 years prior to death were 0.89, 0.61, and 0.11 respectively. This is in contrast to the usual index of exposure, cumulative exposure, in which all exposures are weighted equally.

Benzene has been called a radiomimetic chemical [Parke, 1996]. Like ionizing radiation it can produce bone marrow depression and leukemia. The mechanism is thought to be of oxidative damage to chromosomes induced by benzene metabolites [Snyder and Hedli, 1996], similar to the damage caused by the free radicals generated in the cell by ionizing radiation [Alpen, 1998]. The pattern of leukemia risk after exposure to benzene is similar to that for leukemia following exposure to ionizing radiation, such as observed among the atomic bomb survivors [Darby et al., 1985], patients treated with x-ray for ankylosing spondylitis [Weiss et al., 1995], women given x-ray therapy for metropathia hemorrhagica [Darby et al., 1994], and patients treated with radiation for cancer of the cervix [Boice et al., 1987] and uterine corpus [Curtis et al., 1994]. Figure 1 compares the temporal patterns of leukemia risk following chemical (benzene) and radiation exposure, and the similarities are apparent.

It is common to use “cumulative exposure,” the sum of all exposures incurred, as the exposure metric in exposure–response analyses of occupational and environmental exposures. That was the metric used by Rinsky [Rinsky et al., 1987] and colleagues in their original analysis of the benzene cohort, and was more recently utilized by Schnatter et al. [1996] and Rushton and Romaniuk [1997] in their analyses of leukemia among petroleum distribution workers exposed to benzene. Alternative measures of exposure

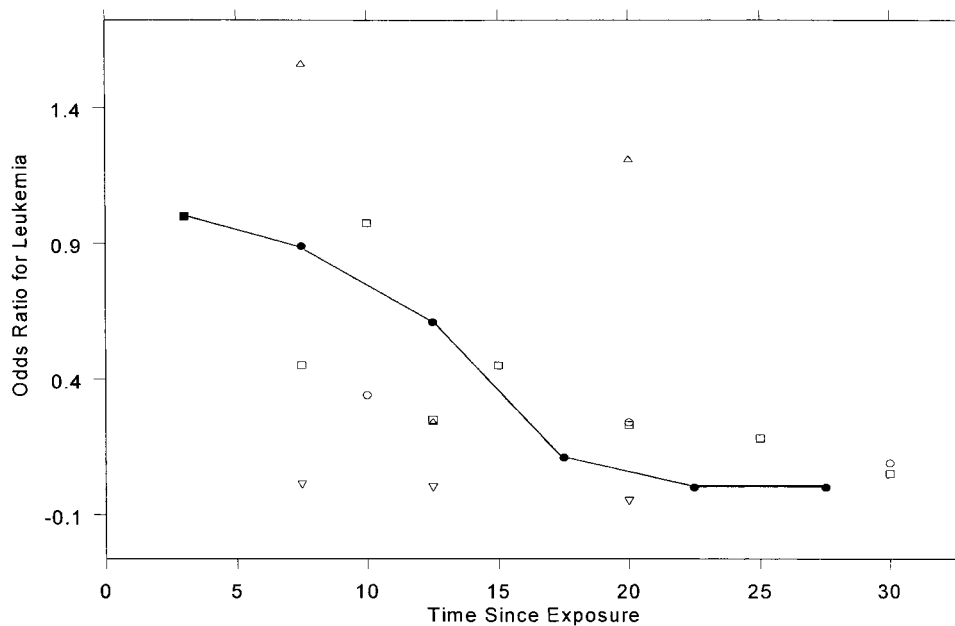


FIGURE 1. Odds Ratios for leukemia in relation to time since exposure for workers exposed to benzene and for subjects exposed to ionizing radiation. The Odds Ratios for each study have been standardized so that the Odds Ratio = 1.0 for the time interval less than 5 years from exposure. The studies are: benzene workers (solid black circle); patients treated with x-ray for ankylosing spondylitis [Weiss et al., 1995] (empty circle); atomic bomb survivors [Darby et al., 1985] (open squares); women treated with radiation for cancer of the uterine corpus [Curtis et al., 1994] (upwards triangle); and, women treated with radiation for cervical cancer [Boice et al., 1987] (downward triangles).

TABLE III. Leukemia Odds Ratios in Relation to Benzene Air Concentration and Exposure Metrics for a 30 Year Exposure Period

Leukemia Odds Ratios			
	Exposure metric: Cumulative exposure	Exposure metric: Benzene exposure index	Exposure metric: Square of benzene exposure index
5 ppm	1.50	1.94	3.76
1 ppm	1.08	1.14	1.30
0.5 ppm	1.04	1.07	1.14
0.1 ppm	1.01	1.01	1.03

considered by those authors included duration of exposure and mean and maximum intensities of exposure. None of those traditional measures account for the temporal patterns of exposure or of risk. That has implications for the evaluation of risk in epidemiologic studies and for the selection of protective standards for workers and the public. In considering occupational exposure standards, Rinsky et al. [1987] computed cumulative exposure for workers exposed to benzene at various air levels for a working lifetime of 40 years. For environmental regulations, cumulative exposures may be computed over a lifetime of 70 years. If remote exposures are less potent than more recent ones with respect to the induction of tumors, then giving equal weight to all exposures will underestimate the potency of the more recent exposures. Table III compares leukemia odds ratios, computed with three statistical models, for a 30-year history of exposure to benzene at various air concentrations. The exposure metrics for these models are cumulative exposure, the BEI, and the square of the BEI. It can be seen that the leukemia risk, at all air concentrations, is higher for the BEIs than for cumulative exposure. Risk assessment using cumulative exposure as the exposure metric will thus underestimate risk.

Recent analyses of specific occupational and environmental carcinogens, including benzene [Hayes et al., 1997] and radon [Lubin et al., 1994] have indicated that risk tends to decline as the time from exposure increases. This suggests that standards for control of occupational or public risk must be selected to control exposures over a narrower time frame than the usual lifetime one. In the case of benzene, it would appear that risk is attributable primarily to exposures incurred during the previous 10 to 20 years, with exposures in the most recent 10 years being the most potent. To limit risk, exposures must be controlled during that interval. Because of the possibility of temporal variability, it is important that epidemiologists explore the temporal pattern of risk among their subjects in the analysis of risk following exposure to other carcinogens. The results of published studies are generally not amenable to reanalysis

from the published tables, and the calculations must be done with the original data.

ACKNOWLEDGMENTS

I thank Robert Rinsky of the National Institute for Occupational Safety and Health who provided me with the benzene data file.

REFERENCES

- Alpen EL. 1998. Radiation biophysics. San Diego: Academic Press.
- Boice JDJ, Blettner M, Kleinerman RA, Stovall M, Moloney WC, Engholm G, Austin DF, Bosch A, Cookfair DL, Krentz ET. 1987. Radiation dose and leukemia risk in patients treated for cancer of the cervix. *J Natl Cancer Inst* 79:1295–1311.
- Curtis RE, Boice JDJ, Stovall M, Bernstein L, Holowaty E, Karjalainen S, Langmark F, Nasca PC, Schwartz AG, Schymura MJ. 1994. Relationship of leukemia risk to radiation dose following cancer of the uterine corpus. *J Natl Cancer Inst* 86:1315–1324.
- Darby SC, Nakashima E, Kato H. 1985. A parallel analysis of cancer mortality among atomic bomb survivors and patients with ankylosing spondylitis given X-ray therapy. *J Natl Cancer Inst* 75:1–21.
- Darby SC, Reeves G, Key T, Doll R, Stovall M. 1994. Mortality in a cohort of women given X-ray therapy for metropathia haemorrhagica. *Int J Cancer* 56:793–801.
- Efron B, Tibshirani RJ. 1994. An introduction to the bootstrap. London: Chapman and Hall.
- Glantz SA, Slinker BK. 1990. Primer of applied regression and analysis of variance. New York: McGraw-Hill Inc.
- Hayes RB, Yin SN, Dosemeci M, Li GL, Wacholder S, Travis LB, Li CY, Rothman N, Hoover RN, Linet MS. 1997. Benzene and the dose-related incidence of hematologic neoplasms in China. Chinese Academy of Preventive Medicine–National Cancer Institute Benzene Study Group. *J Natl Cancer Inst* 89:1065–1071.
- International Agency for Research on Cancer. 1982. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans: some industrial chemicals and dyestuffs. Lyon, International Agency for Research on Cancer. Vol. 29, 93–148.
- Long JS. 1997. Regression models for categorical and limited dependent variables. Thousand Oaks, CA: Sage.
- Lubin JH, Boice JD, Edling C. 1994. Radon and lung cancer risk: a joint analysis of 11 underground miners studies. Bethesda, MD: National Institutes of Health.
- Mathsoft I. S-Plus 2000. 1999. Seattle, WA: MathSoft, Inc.
- NAS/NRC. 1990. Health effects of exposure to low levels of ionizing radiation. BEIR V. Committee on the Biological Effects of Ionizing Radiation. Washington, DC, National Academy Press.
- Parke DV. 1996. Personal reflections on 50 years of study of benzene toxicology. *Environ Health Perspect* 104(Suppl 6):1123–8: 1123–1128.
- Rinsky RA, Smith AB, Hornung R, Fillion TG, Young RJ, Okun AH, Landrigan PJ. 1987. Benzene and leukemia. An epidemiologic risk assessment. *N Engl J Med* 316:1044–1050.
- Rushton L, Romaniuk H. 1997. A case-control study to investigate the risk of leukaemia associated with exposure to benzene in petroleum marketing and distribution workers in the United Kingdom. *Occup Environ Med* 54:152–166.

- Schnatter AR, Armstrong TW, Nicolich MJ, Thompson FS, Katz AM, Huebner WW, Pearlman ED. 1996. Lymphohaematopoietic malignancies and quantitative estimates of exposure to benzene in Canadian petroleum distribution workers. *Occup Environ Med* 53:773–781.
- Sharma S. 1996. *Applied multivariate techniques*. New York: John Wiley and Sons, Inc.
- Snyder R, Hedli CC. 1996. An overview of benzene metabolism. *Environ Health Perspect* 104(Suppl 6):1165–1771.
- SPSS Inc. 1999. Chicago, SPSS Inc.
- Weiss HA, Darby SC, Fearn T, Doll R. 1995. Leukemia mortality after X-ray treatment for ankylosing spondylitis. *Radiat Res* 142:1–11.