Comparison Bias and Dilution Effect in Occupational Cohort Studies

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Health effects of occupational exposures are frequently evaluated by comparing the mortality of a whole cohort of workers with that of the general population. This study design may be affected by two major biases: a dilution effect (DE), due to the inclusion of unexposed subjects in the study cohort, and a comparison bias (CB), due to the different distribution of risk factors in the reference population. A theoretical model of the joint effect of DE and CB is proposed. Their impact was evaluated in two actual cohorts, selecting specific causes of death based on a priori hypotheses of an association. A linear relationship between the risk estimates and the two biases was found after applying either direct or indirect standardization to adjust for confounding. In the two cohorts, higher risks in exposed workers emerged only after adjusting for DE and CB. Cohort studies without an internal referent group may provide unreliable results. *Key words*: bias; cancer; healthy-worker effect; occupational exposure.

In occupational cohort studies, exposure effect should be assessed by comparing exposed workers with at least one unexposed cohort, as similar as possible to the exposed one in all other relevant aspects.[1,2] However, in numerous instances, monitoring data of either early or recent exposure levels are not available, and even information about job categories, which in some cases has been used as a valid surrogate of exposure,[3–5] is not reported. As a consequence, sev-eral occupational cohort studies have compared the total workforce with an external reference population. The consequence of such an approach is frequently a dilution effect (DE), due to the inclusion of unexposed or low exposed subjects in the study cohort.[1] DE is expected to induce a bias toward null, i.e., to reduce the risk estimates, in case of a true positive association. For this reason, the study of large cohorts and meta-analyses may enhance statistical power, allowing the detection of significant effects of exposures even when such bias occurs. However, general populations may differ from occupational cohorts in many respects, including exposures to lifestyle risk factors and health conditions, therefore affecting the estimates of occupa-tional risks, a phenomenon known as comparison bias (CB).[1] Because in many instances occupational cohorts tend to be healthier than general populations of the same age and gender, *CB* is in general due to the healthy worker effect (HWE).[1,6]

The aim of the present study was to estimate the joint impact of DE and CB in occupational cohort studies in a model developed under simple hypothe-ses. The potential impact of such biases is illustrated in two occupational cohorts exposed to recognized carcinogens.

METHODS

Estimating Bias Due to the Joint Effect of DE and CB

The association between exposure and risk for selected diseases in cohort studies is generally assessed by com-puting standardized mortality ratios (SMRs), obtained by indirect standardization, i.e., dividing the observed events in the study cohort by the number expected on the basis of the age-specific mortality rates in a referent (standard) population.[2] The SMR represents a rate ratio between two populations and, in the absence of confounding factors, it may be considered an unbiased estimate of the relative risk (RR) associated with an occupational exposure, provided that either mortality rates are proportional across age classes in the two pop-ulations or they do not differ on age structure.[2]

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Let SMR_W be the SMR for a specific cause, obtained by comparing a hypothetical whole occupational cohort with an external standard population. In the simplest hypothesis, the total cohort may be split into two exposure categories, i.e., exposed and unexposed. Using the same standard population, two different SMRs may be calculated (SMR_E and SMR_{NE}, respectively). Let O_W and E_W be the observed and expected counts of deaths in the whole cohort, respectively, and O_E, E_E, O_{NE}, E_{NE} be the corresponding figures for the two subcohorts. The estimated relative risk, obtained as the whole SMR for the total cohort, will be:

$$SMR_W = \frac{\Sigma\, O_W}{\Sigma\, E_W} = \frac{\Sigma\, O_E + \Sigma\, O_{NE}}{\Sigma\, E_E + \Sigma\, E_{NE}} =$$

$$SMR_E \times \left(\frac{\Sigma\, E_E}{\Sigma\, E_E + \Sigma\, E_{NE}} \right) + SMR_{NE} \times$$

$$\left(\frac{\Sigma\, E_{NE}}{\Sigma\, E_E + \Sigma\, E_{NE}} \right) \quad (1)$$

Then SMR_W is, not surprisingly, the weighted mean between the RRs in the exposed and unexposed subcohorts, whose corresponding weights are the proportions of the expected counts in the two groups. If the two subcohorts do not appreciably differ in age structure (and in the distributions of the other factors used for the standardization procedure, if any), such weights will reflect the proportion of the unexposed subjects. Then, such parameter may be considered to be a measure of dilution effect, which may be defined as:

$$DE = \frac{E_{NE}}{E_{NE} + E_E} \quad (2)$$

Following Kleinbaum et al.,[7] a relative bias may be defined as the difference between the parameter estimated in the "actual" population under study and the corresponding parameter of the target population, divided by the latter. The comparison of a pooled cohort with a general population relies on the implicit assumption that the risk of the latter does not substantially differ from that of unexposed workers. In this framework, CB may be defined as the relative difference between the risk in the unexposed subcohort (R_{NE}) and the risk in the general (standard) population (R_S):

$$CB = \frac{R_{NE} - R_S}{R_S} = \frac{R_{NE}}{R_S} - 1 \quad (3)$$

Using the indirect standardization procedure, the RR between the unexposed subcohort and the general population may be estimated by computing the SMR for the unexposed group (SMR_{NE}). As a consequence, the following definition of CB is obtained from equation 3:

$$CB = SMR_{NE} - 1 \quad (4)$$

Such a definition is consistent, because CB tends to zero when no difference in risk between unexposed workers and the general population exists (i.e., $SMR_{NE} = 1$) and provides a value of 1 when a 100% excess risk occurs (i.e., $SMR_{NE} = 2$).

Furthermore, according to Breslow and Day,[2] under the previously cited conditions of validity for the SMR, the ratio between SMR_E and SMR_{NE} may be considered to be an unbiased estimate of the relative rate, or rate ratio, associated with the exposure under study (RR_E), i.e.:

$$RR_E = \frac{SMR_E}{SMR_{NE}} \quad (5a)$$

and the related exact $100(1-\alpha)\%$ confidence limits of RR_E (RR_{E-L} and RR_{E-U}, respectively) may be obtained by the following equations[2]:

$$RR_{E-L} = \frac{\dfrac{O_E}{O_E + (O_{NE} + 1) \times F_{\alpha/2\,2O_{NE}+2,2O_E}} \times E_{NE}}{\left(1 - \dfrac{O_E}{O_E + (O_{NE} + 1) \times F_{\alpha/2\,2O_{NE}+2,2O_E}}\right) \times E_E}$$

$$RR_{E-L} = \frac{\dfrac{(O_E + 1) \times F_{\alpha/2\,2O_{NE}+2,2O_{NE}}}{O_{NE} + (O_E + 1) \times F_{\alpha/2\,2O_E+2,2O_{NE}}} \times E_{NE}}{\left(1 - \dfrac{(O_E + 1) \times F_{\alpha/2\,2O_E+2,2O_{NE}}}{O_{NE} + (O_E + 1) \times F_{\alpha/2\,2O_E+2,2O_{NE}}}\right) \times E_E}$$
$$(5b)$$

where $F_{\alpha/2,v1,v2}$ are the upper $100_{\alpha/2}$ percentile of the F distribution with v1 and v2 degrees of freedom.

As a consequence, the following relationship between RR_E and SMR_E does exist:

$$SMR_E = RR_E \times (CB + 1) \quad (6)$$

Applying equations 2, 4, and 6 to equation 1, the relationship between the biased SMR for the whole cohort (SMR_W) and the unbiased relative risk between exposed and unexposed workers (RR_E) is obtained as a function of DE and CB:

$$SMR_W = SMR_E \times (1 - DE) + SMR_{NE} \times DE = RR_E \times$$
$$SMR_{NE} \times (1 - DE) + SMR_{NE} \times DE = RR_E \times (1 - DE) \times$$
$$(CB + 1) + (CB + 1) \times DE \quad (7)$$

The same relationship between the biased and the unbiased estimators of relative risk, as a function of DE and CB, was also found in the framework of the direct standardization, as illustrated in the Appendix.

Figure 1A shows the relationship between SMR_W and RR_E when comparison bias does not occur (i.e., when $CB = 0$). As expected, the observed (biased) SMR_W tends to take on the risk for unexposed workers as DE increases.

Figure 1B shows the effect of a $CB = -25\%$ (corresponding to a 25% excess risk in the standard population with respect to the unexposed group) under different hypothetic values of DE. When $DE = 50\%$, approximately corresponding to the inclusion of 50% of unexposed people in a workers cohort, with similar age structure, relative risks less than 1.7 correspond to an SMR_W lower than 1, and a doubling risk due to a hypothetical exposure corresponds to an SMR_W of only 1.13. Moreover, with CB = –25% and DE = 75%, a true RR_E of nearly 2.5 would be required in order for the SMR_W to even reach 1.0, and in actual cohorts a much higher RR_E would be needed to achieve statistical significance.

Finally, figure 1C shows the effect of a $CB = -50\%$, which corresponds to a mortality in the unexposed subcohort reduced to half that in the standard population. When DE exceeds 50%, an SMR_W of 2.0 can be observed only if RR_E is as high as 7.0 or more.

Figure 2 shows the expected 95% confidence intervals (95% CI) of SMR_W, based on the Poisson distribution, for different values of CB and DE. In particular, for a CB = –50% and a DE = 25% (figure 2C), under the assumption of a quite elevated true relative risk for the exposed population (i.e., $RR_E = 3.0$), a sample size of at least 100 observed cases is needed to reach the statistical significance of the corresponding SMR_W. Furthermore, in the presence of a rather low effect of the exposure (e.g., $RR_E = 1.8$) and a high number of observed cases ($O_W = 100$), a statistically significant negative association between the mortality risk and the exposure may emerge ($SMR_W = 0.80$; 95% CI = 0.65;0.97; figure 2C), indicating that, in some instances, the joint effect of CB and DE may induce a reverse bias. It is interesting that similar "protective" effects of occupational exposures are often reported in actual cohorts, in particular for lung cancer mortality. On the contrary, applying equation 5b, after having identified the two groups of truly exposed and unexposed workers, a statistically significant positive effect of the exposure would be observed ($RR_E = 1.8$; 95% CI = 1.05–3.07).

RESULTS

Cancer Mortality in an Italian Plant Manufacturing Vinyl Chloride Monomer and Polyvinyl Chloride

To evaluate the impact of the joint occurrence of *CB* and *DE* in an actual cohort, a reanalysis of cancer mortality in workers employed in a vinyl chloride monomer (VCM) and polyvinyl chloride (PVC) production facility in Northeast Italy is provided below. A mortality follow-up study previously conducted revealed an
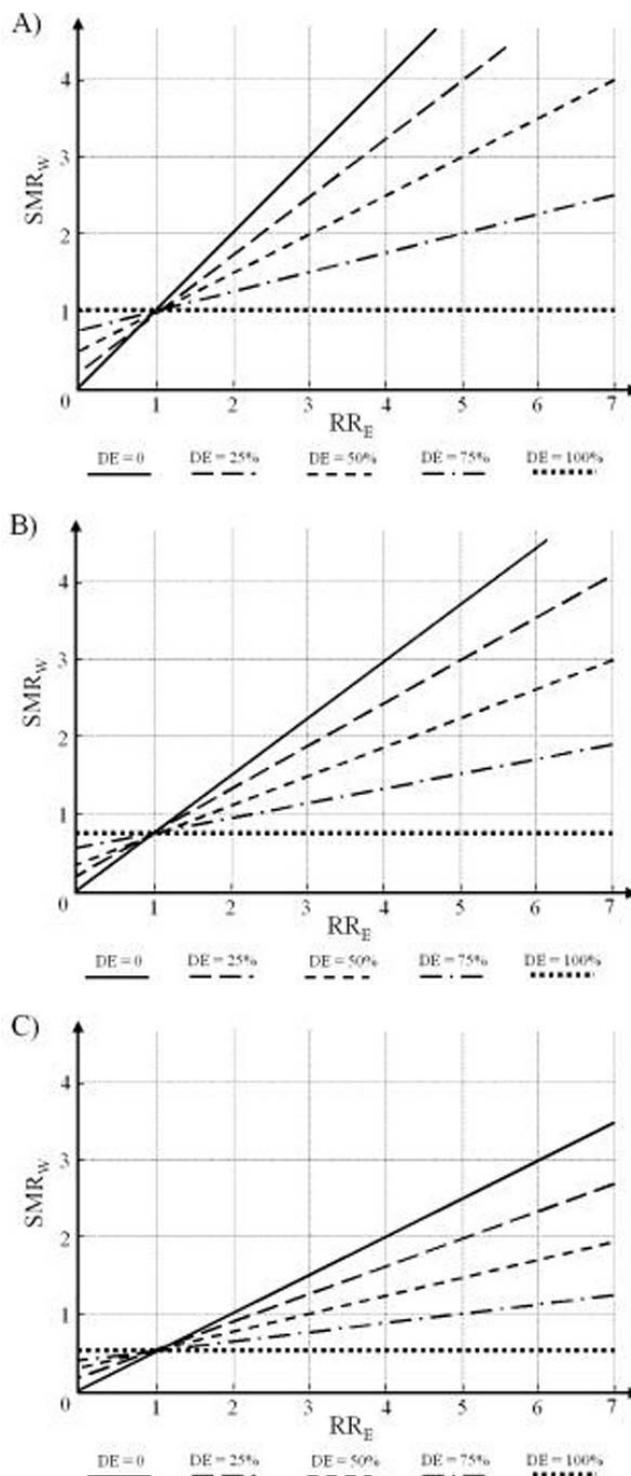

Figure 1—Effects of comparison bias (CB) *and dilution effect* (DE) *on the relationship between the SMR estimated on a whole cohort* (SMR_W) *and the actual relative risk due to the exposure* (RR_E). *Part A: CB = 0; Part B: CB = –25%; Part C: CB = –50%*

excess of liver cancer for the total cohort compared with the general population.[8,9] Further analyses of a subgroup of job categories highly exposed to PVC or VCM (namely: PVC baggers, PVC compound and auto-
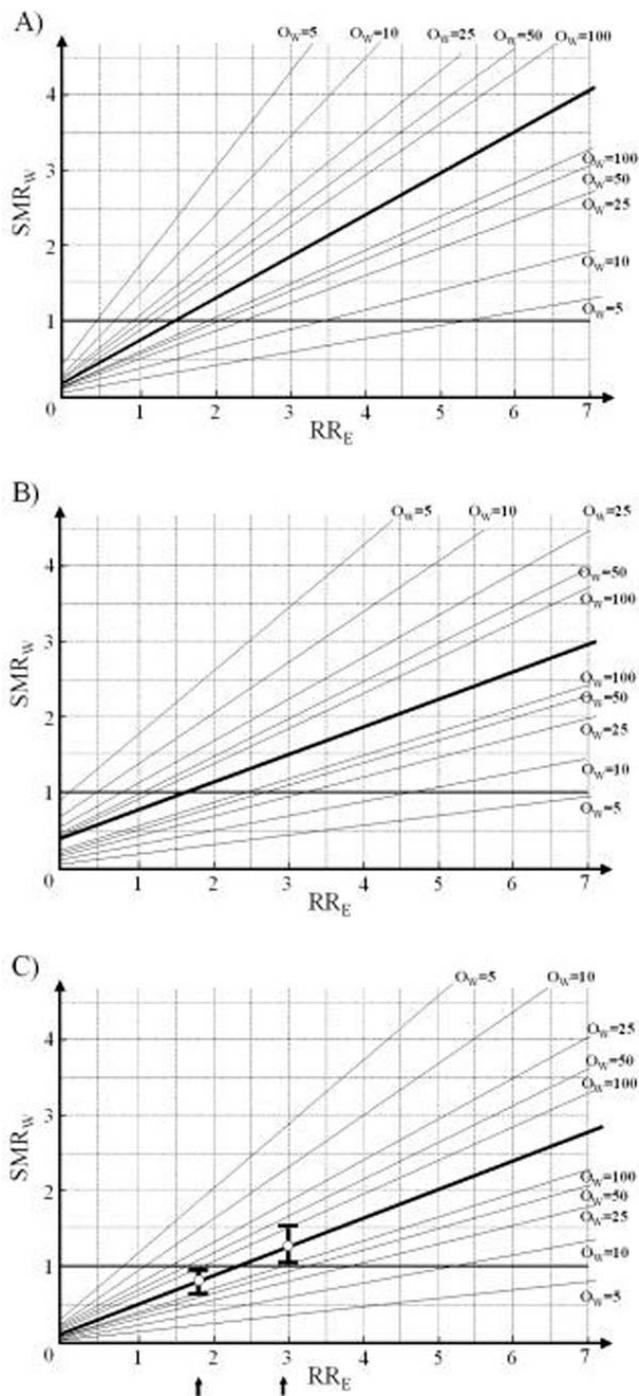
Figure 2—Confidence limits of SMR_W, based on Poisson distribution, as a function of O_W, CB, and DE. Part A: CB = −25%, DE = 25%; Part B: CB = −25%, DE = 50%; Part C: CB = −50%, DE = 25%.

clave workers) was conducted with reference to the unexposed and less exposed workers.[8–10] Elevated risks emerged also for all causes, all tumors, and lung, hemolymphopoietic system, and brain tumors.

The characteristics of the cohort are briefly summarized in Table 1. An update of the follow-up of this cohort is currently in progress. In the present investi-

gation, mortality risk of the whole (pooled) cohort is compared with that obtained by dividing workers into exposed and unexposed subgroups and estimating the relative risk between these two categories (equation 5a). In both analyses the general Italian population was selected as the standard.

The dilution effect, estimated by applying equation 2 to all causes of death combined, was 48.1%. As expected, this value is consistent with the corresponding proportions of both person-years and number of exposed workers under study reported in Table 1 (i.e., 49.5% and 49.1%, respectively).

Table 2 shows the SMRs in the two exposure subcohorts, and the respective 95% CIs, based on Poisson distribution.[2] The SMRs for the unexposed group were all below unity (Table 2A), indicating that comparison bias occurred, with a strong effect for cardiovascular diseases (CB = −65%, Table 2B) and a lower impact for cancer causes (CB = −18%). Among the exposed group, an elevated risk was observed for all causes of interest, and particularly for liver cancer and cancer of the hemolymphopoietic system, with SMRs of approximately 2.8 for both diseases ($p < 0.05$). As regards brain cancer, only two cases were observed, both in the exposed category. Table 2B shows the comparison between risk estimates obtained using the total cohort vs. the general population (SMR_W) and those obtained as the ratio of the SMRs of the exposed and the unexposed groups (RR_E). As expected, RR_E exceeded SMR_W for each cause of death of interest. As regards brain cancer, RR_E was not evaluable, because no case was observed in the unexposed category. Finally, the excess risk observed in the exposed group reversed the negative association among the total cohort ($SMR_W = 0.74$).

Mortality in an Italian Lead-smelting Plant.

The second mortality study was conducted in a lead-smelting plant located in the Sardinia region of Italy. A first report analyzed the 1973–92 mortality by the G6PD-deficient phenotype.[12] Because the cohort was quite young, only very few deaths were observed, and it was possible to analyze only major groups of causes of death. For the purposes of this study, mortality was updated with 11 additional years of follow-up.

Table 3 shows the characteristics of this cohort. As only nine women were included among the white-collar workers, and all of them were alive at the end of follow-up, they were excluded from the study. The available occupational information included only the distinction beween clerks and blue-collar workers. No further distinction was possible within the blue collar workers, such as, for instance, between maintenance and production workers.

Table 4 shows the SMRs for all causes and for selected causes of death in the two subcohorts, when expected events were derived from the general Sardin-

TABLE 1 Characteristics of the PVC Manufacturing Plant Cohort

	White-collar	Autoclave	PVC Baggers	PVC Compound	Other	Total
Number of workers	202	209	197	403	639	1,650
Person-years	4,456	4,588	4,268	8,770	13,543	35,625
Age at hire, mean (SD)	27.4 years (7.1)	26.1 years (5.2)	31.7 years (7.5)	29.4 years (7.2)	29.5 years (8.2)	29.1 years (7.6)
Age at end of follow-up, mean (SD)	56.1 years (8.4)	54.6 years (7.4)	61.0 years (8.0)	57.9 years (7.9)	56.1 years (8.8)	56.9 years (8.5)
Duration of employment, mean (SD)	15.2 years (8.2)	12.7 years (8.1)	11.7 years (8.0)	13.3 years (8.6)	11.7 years (7.5)	12.6 years (8.1)
Entered cohort in years						
1950–59	43 (21.3%	28 (13.4%	31 (15.7%)	74 (18.4%)	122 (19.1%)	298 (18.1%)
1960–69	65 (32.2%	91 (43.5%	116 (58.9%)	172 (42.7%)	135 (21.1%)	579 (35.1%)
1970–79	94 (46.5%	90 (43.1%	157 (25.4%)	157 (39.0%)	367 (57.4%)	758 (45.9%)
1980–95	—	—	—	—	15 (2.3%)	15 (0.9%)

ian male population. In white-collar workers, cancer mortality was less than expected (SMR = 0.63), mostly due to deficits in mortality from lung cancer, bladder cancer, and cancer of the hemolymphopoietic system (Table 4A). Table 4B compares risk estimates in the whole cohort (SMR_W) with those corrected using equation 5a (RR_E). The calculation resulted in an evident increase in lung cancer risk. Risk of cancer of the hemolymphopoietic system also showed a modest increase. Opposite trends emerged for cardiovascular disease and for brain cancer, although the latter was based on one observed case in each group.

DISCUSSION

Cohort studies are considered to be the most appropriate observational investigations to assess causal associations between selected exposures and the risks of developing diseases.[2] However, in occupational settings, the frequent lacks of detailed industrial hygiene measurements, particularly for past years, and often even of the occupational histories of cohort members, are important limits in numerous such investigations. In some cases, the identification of more heavily exposed job categories within an occupational cohort has been demonstrated to be a valid surrogate when industrial hygiene data are missing.[3,5] However, this task requires detailed knowledge about the industrial processes involved, and in numerous occupational investigations only results from the analyses of a cohort as a whole are provided. In such a study design the joint occurrence of DE and CB may induce a strong underestimate of risk for selected diseases, as shown in simulated data in figure 1, based on equation 7. As shown in the Appendix, the effect of the two types of bias is sim-

ilar when using direct standardization to control for age and other possible confounders (equation A.7). In a similar way, results are expected to be biased when applying multivariate regression analysis. For instance, the Poisson regression model, while making it possible to control for a set of possible confounders, is equivalent to either indirect or direct standardization procedures, in that either expected counts or populations at risk are included as offsets.[2] As a consequence, not even this widely applied analytical approach can reduce the impact of the two types of bias, unless the truly exposed subcohort was correctly identified.

In actual occupational cohorts, the extension of DE and CB should not be considered as negligible. As regards DE, the proportion of unexposed may vary depending on the type of exposure, physical status, and preventive measures available in the occupational environment (indoor/outdoor work, ventilation systems, use of protective masks and gloves). In the examples considered above, DEs ranged from 49% in the PVC cohort to 24% in the lead-smelter cohort. Even if these might seem to be two extreme examples, in some instances even larger DEs have been reported. For example, in both ten U.S.[13] and one Italian[3] petrochemical cohorts, job categories unexposed to asbestos included more than 60% of workers.

As regards CB, according to equation 4 it may vary from –100% to infinite, but it is likely to assume mainly negative values, being in general strictly associated with the HWE.[1] The HWE is made up of two major factors, an initial component called the "healthy-worker hire effect" (HWHE), due to a greater chance for healthy subjects to gain employment, and a continuing component called the "healthy-worker survivor effect" (HWSE), mainly due to the higher probability for

TABLE 2 Mortality in the PVC Manufacturing Plant Cohort

A. Number of Observed Deaths (Obs) and Standardized Mortality Ratio (SMR) by Exposure Category and Cause of Death

Cause of Death (ICD9)	Exposed Categories			Unexposed Categories		
	Obs	SMR	95% CI	Obs	SMR	95% CI
All causes (0–999)	107	0.91	0.74–1.09	63	0.58	0.44–0.74
Cardiovascular diseases (390–459)	18	0.49	0.29–0.77	12	0.35	0.18–0.62
All cancers (140–208)	54	1.20	0.90–1.57	34	0.82	0.57–1.15
Liver cancer (155)	9	2.83	1.29–5.37	2	0.68	0.08–2.44
Lung cancer (162)	20	1.25	0.77–1.94	11	0.75	0.38–1.35
Brain cancer (191)	2	1.43	0.17–5.16	0	0.00	0.00–2.79
Hemolymphopoietic system cancer (200–208)	7	2.78	1.01–5.72	2	0.84	0.10–3.10

B. Comparison between the SMR of the Whole Cohort (SMR_W) (Standard: General Population), and the Risk Estimate Obtained from Selecting an Internal Referent Group of Unexposed Workers (RR_E)*

Cause of Death (ICD9)	CB %	DE %	SMR_W	95% CI	RR_E	95% CI
All causes (0–999)	−42	48	0.75	0.64–0.87	1.57	1.14–2.18
Cardiovascular diseases (390–459)	−65	48	0.42	0.29–0.60	1.38	0.63–3.13
All cancers (140–208)	−18	48	1.02	0.82–1.25	1.47	0.94–2.32
Liver cancer (155)	−32	48	1.79	0.89–3.20	4.16	0.87–39.8
Lung cancer (162)	−25	48	1.02	0.69–1.31	1.67	0.76–3.86
Brain cancer (191)	−100	49	0.74	0.09–2.66	n.e.	—
Hemolymphopoietic system cancer (200–208)	−16	49	1.83	0.84–3.48	3.31	0.63–32.75

*CB = comparison bias; DE = dilution effect; n.e. = not evaluable.

healthy subjects to maintain the same occupation.[6,14] Furthermore, other factors such as routine disease screening and physical exercise might also contribute to the HWSE.[1] CB is expected to be more relevant for non-neoplastic diseases and to tend to decrease with increasing time from hire. However, many occupational studies report clear negative CBs for many cancer sites also. For example, a large meta-analysis of cohort studies of petrochemical workers reported a statistically significant lower risk for all cancers combined (SMR = 0.86; 95% CI = 0.85–0.88) as well as for many considered sites, including lung (SMR = 0.81; 95% CI = 0.79–0.83) and bladder (SMR = 0.78; 95% CI = 0.71–0.85).[15] Furthermore, an analysis including more than 58,000 nuclear workers in France showed a significant lower risk of cancer mortality compared with the national population.[16] The deficit was limited to male workers and included many specific malignancies, including lung cancer (SMR = 0.68; 95% CI = 0.62–0.74) and lymphohematopoietic neoplasms (SMR = 0.83; 95% CI = 0.71–0.96).[16] Safety prescriptions in the occupational environment, such as restriction of smoking during working hours, may play some role in the HWSE component for cancer mortality, which is expected to remain lower than in general population after a long observation period. Moreover, a recent prospective cohort study of agricultural workers in the United States, reported a deficit in lung cancer mortality compared with the general population in the smokers subcohort also, suggesting that factors other than a low tobacco consumption may play some protective role.[17] Finally, HWE may be influenced by many other factors, including race, gender, age at hire, occupational class, and socioeconomic conditions, and their

TABLE 3 Characteristics of the Lead Smelter Cohort

	Blue-collar	White-collar	Total
Number of workers	1,015	329	1,344
Person-years	23,842.84	8,651.26	32,494.10
Age at hire, mean (SD)	31.6 (8.6)	32.1 (7.4)	31.7 (8.4)
Age at end of follow-up, mean (SD)	54.2 (11.1)	57.5 (8.1)	55.0 (10.5)
Duration of employment, mean (SD)	10.1 (7.0)	13.0 (6.5)	10.8 (7.0)
Entered cohort in years			
Jan 1, 1973	263 (25.9%)	158 (48.0%)	421 (31.3%)
1973–1980	394 (38.8%)	103 (31.3%)	497 (37.0%)
1981 onward	358 (35.3%)	68 (20.7%)	426 (31.7%)

TABLE 4. Mortality in the Lead-smelters Cohort

A. Number of Observed Deaths (Obs) and Standardized Mortality Ratio (SMR) by Exposure Category and Cause of Death

Cause of Death (ICD9)	Blue-collar Workers			White-collar Workers		
	Obs	SMR	95% CI	Obs	SMR	95% CI
All causes (0–999)	117	0.75	0.63–0.90	23	0.52	0.33–0.78
Cardiovascular diseases (390–459)	26	0.53	0.36–0.77	10	0.79	0.38–1.45
All cancers (140–208)	47	0.94	0.69–1.26	10	0.63	0.30–1.16
Stomach cancer (151)	3	1.12	0.23–3.27	1	1.24	0.02–6.88
Lung cancer (162)	16	1.05	0.60–1.71	2	0.39	0.05–1.41
Bladder cancer (188)	3	1.54	0.32–4.50	0	0.00	0.00–6.96
Brain cancer (191)	1	0.87	0.02–4.85	1	2.46	0.06–13.69
Hemolymphopoietic system cancer (200–208)	5	1.29	0.42–3.01	1	0.81	0.02–4.53

B. Comparison between the SMR of the Whole Cohort (SMR$_W$) (Standard: General Population), and the Risk Estimate Obtained from Selecting an Internal Referent Group of Unexposed Workers (RR$_E$)*

Cause of Death (ICD9)	CB %	DE %	SMR$_W$	95% CI	RR$_E$	95% CI
All causes (0–999)	–48	22	0.68	0.57–0.79	1.45	0.91–2.36
Cardiovascular diseases (390–459)	–21	20	0.56	0.39–0.78	0.66	0.32–1.39
All cancers (140–208)	–35	24	0.87	0.66–1.12	1.50	0.75–3.32
Stomach cancer (151)	+24	23	1.15	0.31–2.93	0.90	0.07–47.6
Lung cancer (162)	–61	25	0.90	0.53–1.40	2.69	0.63–24.2
Bladder cancer (188)	–100	21	1.21	0.25–3.54	n.e.	—
Brain cancer (191)	+146	26	1.28	0.16–4.63	0.35	0.01–27.8
Hemolymphopoietic system cancer (200–208)	–16	24	1.17	0.43–2.55	1.59	0.18–75.0

*CB = comparison bias; DE = dilution effect; n.e. = not evaluable.

different impacts on different occupational cohorts suggest that their mechanisms of action have not been completely clarified.[6]

In both analyzed cohorts, CB emerged for almost each considered cause of death. In the PVC facility workers, comparing the risk estimates calculated for the total pooled cohort with that obtained adjusting by an internal reference group resulted in an elevated, though still not significant, risk for lung cancer, and in more elevated risk estimates for liver cancer and cancer of the hematopoietic system. The excess of liver cancer mortality in workers exposed to VCM is well established.[9] Autoclave workers are probably the category most heavily exposed to this substance, even if VCM may be present as a contaminant in PVC dust.[8–10] As regards lung cancer, exposure to PVC, especially in baggers and, to a lesser extent, the other two categories, may explain the excess risk observed.[18] A higher smoking rate as an alternative explanation for the excess risk of lung cancer in the exposed subcohort cannot be completely ruled out. However, the job categories selected as the internal comparison also showed a risk for lung cancer lower than that for the general population after restricting the analysis to blue-collar workers, whose socioeconomic condition and related lifestyle behaviors are expected to be similar to those of the workers in the exposed group[6] (data not shown).

In the lead-smelter cohort, the joint effect of CB and DE emerged as an important cause of underestimation, especially for lung cancer mortality. The HWE, a possibly low prevalence in the smoking habit among these workers (as suggested by the SMR for nonmalignant respiratory diseases among blue-collar workers, which was 1.12, 95% CI = 0.59–2.13), had contributed to mask the increased risk. An increase in lung cancer risk has been repeatedly reported among workers in lead smelters,[19,20] although the exact causative agent is still unclear. Exposures to known lung carcinogens, such as asbestos, arsenic, cadmium, and polycyclic aromatic hydrocarbons, is typical in lead-smelting plants. An excess risk of liver cancer was associated with exposure to welding fumes and inorganic dust in a case–control study.[21] As lead nitrate is used as a promoter of liver hyperplasia in experimental investigations,[22] future studies specifically addressing the hypothesis of an association are warranted. Mortality from hemolymphopoietic cancer was not elevated among workers in lead smelters and battery workers in a cohort study,[19] while excesses were found in a plant producing lead chromate pigments[23] and in a proportionate mortality study of plumbers and pipefitters.[24] Many cohort studies have reported an excess risk for stomach cancer in workers exposed to lead,[25] which was not confirmed in the present investigation. Moreover, a case–control study nested in a cohort of U.S. workers failed in finding any clear association.[26]

The small sizes of the two cohorts under study resulted in wide confidence intervals of the estimated risks. However, the purpose of the investigation was to estimate the impact of the combined effect of CB and DE in occupational cohort studies. Interestingly, such an effect emerged in both the considered cohorts for almost all selected causes of deaths, which were a priori known, or at least suspected, to be associated with carcinogenic substances in the related occupational environments. Furthermore, for some causes of death (namely, brain cancer in the vinyl chloride industry and lung cancer in the lead-smelting plant), pooled analyses provided risks lower than those of the referent populations.

As single positive studies are not considered proof of a positive association per se, likewise negative studies should not be used as proof of lack of an effect.[1,27,28] Unfortunately, epidemiologic studies containing biases of the above-described magnitude seem to be acceptable within the research community when the consequence is a negative or diminished finding, but their results are considered objectionable when the estimated risks for the exposed population are exaggerated. Moreover, results from investigations on large occupational cohorts have often been used to claim no excess risk for the health of workers, particularly at the presumed low-level concentrations of pollutants in present or recent work environments.[29–34] It should be noted that in many instances the very high statistical power sometimes claimed by the authors of such investigations[35,36] may also be misleading, as the derived estimates might have overlooked the occurrence of a combined CB and DE effect, which, in some instances, may even induce a reverse bias.

Many reasons have been produced to explain the widespread practice of analyzing pooled cohorts of exposed and unexposed workers, even when surrogate measures of exposure could be derived (e.g., from job categories). Even if in some instances a clumsy use of epidemiologic methods can simply be advocated,[1] the large number of investigations funded by industrial companies have raised the suspicion that conflict of interest might play a crucial role.[1,4,37] Finally, negative results from these studies might also be incorrectly extended to suggest no risk for the public exposed to the same chemicals, as exposure levels are greater among workers, thus raising doubts and confusion in both the scientific community and the public.[4,27,28,37]

In conclusion, negative results from the analyses of total cohorts should be accepted with caution, as it is plausible that a substantial fraction of such consist of workers unexposed to the occupational risk factor of interest, which causes DE to occur, strongly enhancing the effect of even a small CB. As CB is not a bias toward null, its occurrence may also prevent the finding of effects of risk factors in studies involving very large numbers of cases. Furthermore, such a bias is likely to be particularly insidious for diseases that have low attributable risks, such as benzene exposure and the risk of leukemia. For these reasons, despite their wide application in epidemiologic literature, results from occupational cohort studies without internal referent groups may be unreliable.

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APPENDIX

Estimating the Bias Due to the Joint Effect of DE and CB Using Direct Standardization

Applying the direct-standardization approach, comparison between mortality for a specific cause in the whole cohort and that of the general (standard) population may be performed computing the comparative mortality fraction (CMF_W), obtained by the ratio between the directly standardized rate in the whole cohort (TST_W) and the corresponding rate of the standard population (TS):

$$CMF_W = \frac{TST_W}{TS} = \frac{\sum_i \frac{O_{w,i}}{m_{w,i}} \times S_i}{\sum O_{S,i}} \qquad (A.1)$$

where i are the age classes, $O_{S,i}$ the deaths observed in the standard population, $m_{w,i}$ and S_i the person-years at risk in the

whole cohort under study and in the standard population, respectively.

Let O_E, and O_{NE} be the observed counts in the exposed and unexposed workers, respectively, and m_E and m_{NE} the corresponding person-years at risk. Define as dilution effect (DE_i) the proportion of person-years of unexposed workers within each age stratum i:

$$DE_i = \frac{m_{NE,i}}{m_{W,i}} = \frac{m_{NE,i}}{m_{NE,i} + m_{E,i}} \qquad (A.2)$$

Note that the definition in the equation A.2 is consistent with that provided in equation 2, obtained using the indirect-standardization approach, the two formulas being equivalent when applied to a single age class.

Considering that $O_{w,i}$ is the sum of $O_{E,i}$ and $O_{NE,i}$, and applying equation A.2 to the equation A.1, the following relationship is obtained:

$$CMF_W = \frac{\sum_i \frac{O_{w,i}}{m_{w,i}} \times S_i}{\sum_i O_{S,i}} = \frac{\sum_i \frac{O_{E,i}}{m_{E,i}} \times \frac{m_{E,i}}{m_{E,i} + m_{NE,i}} \times S_i}{\sum_i O_{S,i}}$$

$$+ \frac{\sum_i \frac{O_{NE,i}}{m_{NE,i}} \times \frac{m_{NE,i}}{m_{E,i} + m_{NE,i}} \times S_i}{\sum O_{S,i}}$$

$$= \frac{\sum_i \frac{\lambda_{E,i}}{\lambda_{S,i}} \times (1 - DE_i) \times O_{S,i}}{\sum_i O_{S,i}} + \frac{\sum_i \frac{\lambda_{NE,i}}{\lambda_{S,i}} \times DE_i \times O_{S,i}}{\sum_i O_{S,i}} \qquad (A.3)$$

where $\lambda_{E,i}$, $\lambda_{NE,i}$, and $\lambda_{S,i}$ are the age-specific rates for the exposed, the unexposed, and the standard populations respectively.

The calculation of an age-adjusted estimate of relative risk relies on the implicit assumption that age may act as a confounder, but it does not modify the effect of the exposure under study. Under this common hypothesis, each age-specific rate ratio may be considered to be an estimate of the relative risk for the whole population under analysis. In particular, the unbiased relative risk (RR_E) due to the exposure may be defined as follows:

$$RR_E = RR_{E,i} = \frac{\lambda_{E,i}}{\lambda_{NE,i}} \qquad (A.4)$$

Furthermore, according to the same hypothesis of equality of the relative risks across the age classes, and according to the definition of comparison bias (CB) reported in equation 3, the following re-definition of CB may be obtained:

$$CB = \frac{R_{NE}}{R_S} - 1 = \frac{\lambda_{NE,i}}{\lambda_{S,i}} - 1 \qquad (A.5)$$

Finally, applying equations A.4 and A.5 to equation A.3, the relationship between the biased and the unbiased estimates of relative risk (CMF_W and RR_E, respectively) is obtained as a function of DE_i and CB:

$$CMF_W = \frac{RR_E \times (CB+1) \times \sum_i (1-DE_i) \times O_{S,i}}{\sum_i O_{S,i}}$$
$$+ \frac{(CB+1) \times \sum_i DE_i \times O_{S,i}}{\sum_i O_{S,i}} \quad \text{(A.6)}$$

If the proportion of exposed workers does not differ by age class, the dependence of CMF_W by the observed cases in the standard population ($O_{S,i}$) disappears:

$$CMF_W = RR_E \times (CB+1) \times (1-DE) + (CB+1) \times DE \quad \text{(A.7)}$$

where $DE = DE_i$ for each i.

Comparing equation A.7 and equation 7, it is evident that the same relationship between the biased and the unbiased estimates of relative risk does exist, applying both the direct or the indirect standardization method to adjust for age (and for other confounders, if any).

More generally, DE_i might vary across the age strata i. For example, some exposures might cause a progressive worsening in general health condition, making easier the transfer of some workers to less exposed or unexposed jobs, especially with increasing age. As a consequence, in some instances, DE_i might be positively correlated to age. On the contrary, some heavy exposures may cause the early retirement of some workers, reducing DE_i in the last age classes. In any case, let $g(i)$ be the unknown function linking DE_i to the age class i:

$$DE_i = g(i)$$

Also let DE_k be a positive value of DE for the $i = k$ age class. A function $f(i)$ may be defined as follows:

$$f(i) = \frac{g(i)}{DE_K} \quad \text{(A.8)}$$

Applying equation A.8 to equation A.6, the following general relationship between CMF_W and RR_E is obtained:

$$CMF_W = RR_E \times (CB+1) \times (1-DE^*)$$
$$+ (CB+1) \times DE^* \quad \text{(A.9)}$$

where:

$$DE^* = DE_K \times \frac{\sum g(i) \times O_{S,i}}{\sum O_{S,i}}$$

Comparing equations A.9 and A.7, a similar relationship between the biased (CMF_W) and unbiased (RR_E) risk estimators also emerges under more general assumptions (i.e., letting DE be dependent by age).