CORRESPONDENCE

Risk of myelogenous leukaemia and multiple myeloma in workers exposed to benzene

Editor—The recent report by Wong presents valuable new analyses concerning benzene and lymphohaematopoietic cancers among workers in the Pliofilm cohort.[1] In spite of a series of analyses of this population,[2–6] no previous analyses have provided specific risk estimates for acute myeloid leukaemia (AML) although the cohort has been widely viewed as providing evidence most pertinent to that outcome. The effort to examine patterns associated with leukaemia subtypes is clearly worthwhile, for the reasons presented by Wong.[1]

However, we would like to challenge two aspects of this report: (*a*) the claim that "Specificity is one of the major criteria for causation analysis" (page 383); and (*b*) the assertion that "by lumping all cell types into a single category, the misconception that benzene can increase the risk of other cell types of leukaemia may be created." (page 382).

Specificity was proposed by Hill some years ago as one of several considerations in evaluating causality,[7] but even then with strong caveats: "We must not, however, overemphasise the importance of the characteristic" (page 297). Subsequent experience and evolution of epidemiological methods has led to virtual abandonment of this as a useful criterion for causality[8] except insofar as it suggests a pattern of bias in self reported exposure data or incomplete follow up for disease. Given the established multiplicity of consequences of ionising radiation, tobacco smoke, asbestos, oral contraceptives, physical activity, and fruit and vegetable consumption, for example, it is actually rather difficult to identify any biologically active exposure that *is* specific in its consequences.

With inferences from the Pliofilm cohort pertaining to leukaemias other than AML, Wong correctly asserts that the numbers of cases of individual cell types are so small as to preclude meaningful analysis, but the number of total non-AML cases (admittedly, a heterogeneous group) is sufficient to analyse. To examine whether the association between benzene exposure and total leukaemia observed previously in this cohort[5] is driven by AML cases, we integrated the results from the two reports (table).[1 5]

These data indicate that the association is stronger for AML than for total leukaemia, but the differences in association for AML, non-AML, and total leukaemia are modest. Also, movement of a single case from AML to another cell type would considerably diminish this pattern and movement of two cases would obliterate it, which serves as a reminder of just how imprecise these standardised mortality ratios are. Wong emphasises the distinction in dose-response patterns found for AML *v* total leukaemia, but the numbers of cases available from this study preclude making such subtle distinctions. Given these results, the claim that they point specifically towards AML as the only type of leukaemia associated with benzene exposure in this cohort is unwarranted.

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Author's reply—Savitz and Andrews raised two points about my recent paper "Risk of acute myeloid leukaemia and multiple myeloma in workers exposed to benzene."[1] Firstly, they questioned whether specificity of disease should be included as a criterion for causation analysis. Secondly, they argued that the data from the Pliofilm cohort indicated that exposure to benzene could result in an increased risk of leukaemia cell types as well as acute myeloid leukaemia (AML).

With regard to the first point, Savitz and Andrews might have misunderstood what "specificity" means. Specificity of disease refers to being "distinct," and does not imply "non-multiplicity" or "exclusiveness." In my paper, I did not claim that because benzene can cause AML, therefore benzene cannot cause other types of leukaemia or other diseases. Certainly we know that, given sufficient exposure, benzene can cause both AML and aplastic anaemia. However, both AML and aplastic anaemia are *specific* (or distinct) diseases. Therefore, specificity does not contradict multiplicity, as long as the diseases involved are specific and meaningful diagnostic entities.

To support their first point, Savitz and Andrews cited a statement in Hill's 1965 paper on causation that we must not over emphasise the importance of specificity.[2] What Savitz and Andrews have omitted from their citation is the example given by Hill on this issue. Hill provided an example that occupational exposure to nickel can cause lung as well as nasal cancer (page 297). Again, both lung and nasal cancers are specific diagnostic entities recognised by the medical professions. Thus, Hill's paper was hardly an endorsement for the practice of combining heterogeneous disease categories for statistical analysis.

Most importantly, specificity of disease is not a statistical issue, but should be based on the biology of the disease. It makes little sense to lump different diseases into a single category for causation analysis. Before any statistical analysis, one must review and consider the underlying biological mechanisms of the disease process. As stated in my paper, it has become increasingly evident that the diseases collectively known as leukaemia are several distinct malignancies.[3–5] Based on their letter, Savitz and Andrews themselves seem to recognise the heterogeneity of various leukaemia cell types. Given this recognition, it is not clear why Savitz and Andrews would endorse combining heterogeneous leukaemia cell types for analysis.

With regard to the second point, Savitz and Andrews provided a risk estimate for non-AML. Such a statistical analysis is meaningless, as non-AML is not a recognised diagnostic entity. Although Savitz and Andrews themselves admitted that such a category was heterogeneous, they nevertheless justified such a statistical analysis on the ground that "the number of non-AML cases in aggregate (admittedly, a heterogeneous group) is sufficient to analyse." Mere sufficiency of cases does not validate an analysis. Any statistical analysis which totally disregards our current understanding of the underlying biological mechanisms is meaningless.

Finally Savitz and Andrews concluded: "the claim that these results (results which I provided in my paper) point specifically toward AML as the only type of leukaemia associated with benzene exposure in this cohort (the Pliofilm cohort) is unwarranted." I did not claim in my paper that the data from the Pliofilm cohort showed that benzene did not cause other types of leukaemia besides AML. I simply stated the following: "For cell types other than AML, the Pliofilm study does not provide sufficient cases for any meaningful analysis. The specific cell type with the second largest number of cases in the Pliofilm study was chronic myeloid leukaemia (CML), consisting of only two deaths. One of the two deaths from CML was employed at the plant for one month in 1948, and died two years later in 1950 at the age of 29. His cumulative exposure was 0·10 ppm-years. Clearly this case could not have been associated with exposure at the plant." Therefore, the Pliofilm study offers little useful information on the relation between benzene exposure and leukaemia cell types other than AML.

The evidence for the lack of an association between benzene and other leukaemia cell types comes from recent laboratory investigations[6–8] and other

Results for AML, non-AML, and total leukaemia in Pliofilm cohort study

Cause of death	*Observed deaths*	*Expected deaths*	*SMR (95% CI)*
Total leukaemia	14	3·89	3·60 (1·97–6·04)
AML	6	1·19	5·03 (1·84–10·97)
Non-AML	8	2·70	2·96 (1·25–5·84)

epidemiological studies,[9-14] which were cited in my previous paper. Laboratory investigations have shown that benzene in vivo and hydroquinone (a benzene metabolite) in vitro alter the recruitment or stimulation of myeloid progenitor cells, thereby increasing the number of cells at risk of developing leukaemia. Furthermore, this effect is selective for myeloid cells only.[8] Similarly, epidemiological cohort and case-control studies based on analyses of specific leukaemia cell types did not detect any increased risk of other leukaemia cell types in people exposed to benzene.[9-14] None of the evidence from these laboratory or epidemiological investigations was considered by Savitz and Andrews.

In my paper[1] I provided an account of why different leukaemia cell types were combined for analysis in some epidemiological studies in the past and why such an analysis would not be appropriate or meaningful given recent developments in laboratory and epidemiological research on the subject. In particular, I showed that ignoring the heterogeneity of leukaemia cell types would underestimate the risk of AML at high concentrations of benzene exposure. Although these high exposures are uncommon in developed countries, in some parts of Asia and eastern Europe they have not been totally eliminated. Advocating an analysis which ignores the underlying biological mechanisms of the disease on one hand and underestimates the risk of occupational exposure on the other is a disservice to the workers.

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Comment on EQM Testmate OP cholinesterase kit

Editor—We read with interest the report by London *et al*, on the EQM Testmate OP cholinesterase kit.[1] We have previously reported good reproducibility of repeated measurements of haemoglobin adjusted erythrocyte cholinesterase with this kit under field conditions.[2] The Pearson correlation coefficient for duplicate measurements on the 23 samples in that study was 0·93 (in a reanalysis of data from our previous study). The correlation between duplicate erythrocyte cholinesterase measurements conducted recently on 20 blood samples, with a production model of the field kit, was 0·98 (Amaya A, unpublished data). In epidemiological studies, the kit has been found to be sensitive to subtle differences in cholinesterase activity based on exposure.[3]

We do not offer an explanation for the poor performance of the kit, in the study of London *et al*, under conditions apparently similar to those under which our evaluation showed good repeatability. However, we recently have found that the temperature adjustment of cholinesterase activity by the field kit can be a source of significant error. In one experiment, we measured haemoglobin adjusted erythrocyte cholinesterase activity according to the manufacturer's specifications, at different ambient temperatures, on blood from the same unexposed person. A sample of blood was drawn in heparin the night before testing and stored at 4°C overnight in six separate aliquots. Each aliquot was defrosted 30 minutes before testing. Figures 1 and 2 suggest that the temperature adjustment by the kit is not accurate, as measured activity on the same sample varied considerably with temperature both for plasma and for erythrocyte cholinesterase.

In another experiment, we examined the rate of thermal equilibration of the kit by moving a Testmate kit from a cool room, where the kit's internal thermometer and an independent external thermometer read 8°C, to a room where the external thermometer read 40°C. The internal thermometer of the kit (the basis for the colorimeter's temperature adjustment) registered only 30°C after 120 minutes at ambient temperature of 40°C. Moving the kit from an air conditioned vehicle to a non-air conditioned field site has been, in our experience, a common scenario. Such rapid changes in temperature immediately before measuring cholinesterase would result in considerable error.

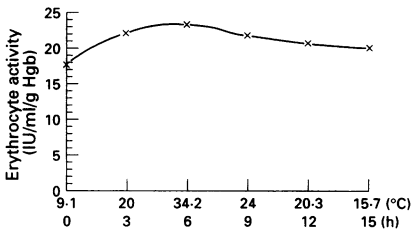

Figure 1 Temperature-adjusted erythrocyte cholinesterase activities as a function of temperature, as measured by the EQM Testmate OP kit.

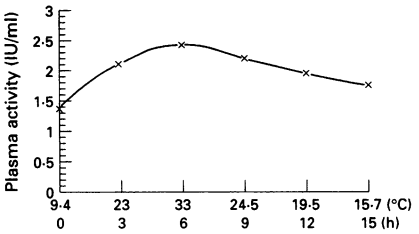

Figure 2 Temperature-adjusted plasma cholinesterase activities as a function of temperature, as measured by the EQM Testmate OP kit.

Although we have found repeated cholinesterase measurement to be highly reliable, in contrast to the experience of London *et al*, we think that variable temperatures under field conditions result in significant error in the accuracy of cholinesterase measurement by the kit. This problem might be solved if the manufacturers were to provide accurate temperature adjustment factors, and if they were to measure directly the temperature of the reagent solution, rather than the temperature of the colorimeter.

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NOTICES

2nd Advanced Course in Occupational and Environmental Epidemiology: Principles of Exposure Assessment. 17–22 June, 1996. The Netherlands.

The Department of Epidemiology and Public Health, and Department of Air Quality, University of Wageningen, The Netherlands, in collaboration with The Netherland Institute for Health Sciences (NIHES) have organised this course. The course is divided in plenary morning sessions and parallel afternoon sessions. The parallel sessions include two modules: module 1: environmental epidemiology; module