Review

Problems in Assessing the Relative Predictive Value of Internal Markers *versus* External Exposure in Chronic Disease Epidemiology[1]

Kyle Steenland,[2] James Tucker, and Alberto Salvan

National Institute for Occupational Safety and Health, Cincinnati, Ohio 45226 [K. S., A. S.]; Biomedical Sciences Division (L-452), Lawrence Livermore Laboratory, Livermore, California 94551 [J. T.]

Abstract

Epidemiology traditionally has relied on measures of "external" exposure in determining the association between exposure and disease. Recently, there has been increasing reliance on internal markers reflecting internal dose and/or early stages of disease. In the context of observational studies of chronic disease in which there is a known exposure-disease association, the question arises whether the external exposure or the internal marker is a better predictor of eventual disease outcome. Here we describe some simple approaches to evaluate the relative predictive value of the internal marker (or biomarker, defined in the most general sense) *versus* the exposure, as well as their limitations. The problems of assessing the predictive value of internal markers for chronic disease are illustrated via two examples: (*a*) carcinogens, cytogenetic outcomes, and cancer; and (*b*) asbestos, asbestosis, and lung cancer. We conclude that it is unlikely that observational epidemiology will allow a full assessment of the predictive value of cytogenetic outcomes *versus* exposure for cancer in humans exposed to known carcinogens in the near future, although animal studies could provide important complementary information. For asbestos, data to date indicate that the presence or absence of asbestosis is a better predictor of lung cancer in an exposed population than is the level of exposure to asbestos itself. In general, the most useful markers for predicting chronic disease are ones which persist over time.

Introduction

Epidemiology traditionally has relied on measures of "external" exposure in determining the association between exposure and disease. For example, in the study of lung cancer the principal exposure of interest has been a history of smoking. Increasingly, epidemiology has been attempting to use internal markers which may be viewed as either a measure of internal dose or early markers of disease. In this article we will refer to these internal markers as biomarkers, defined in the general sense to include not only biological materials measured in tissues (such as DNA adducts or urinary cotinine) but also general measurements of health status such as lung function, left ventricular hypertrophy, or the presence or absence of lung fibrosis.

The hope is usually that the biomarker will be able to better predict eventual disease than the original external exposure, possibly because the marker represents a step on the causal pathway from exposure to disease or possibly because it is simply more associated with the eventual disease outcome than the measure of external exposure, even though it is not on any causal pathway. For example, among smokers one might measure urinary cotinine, AHH[3] enzyme induction, red cell hemoglobin adducts, SCE in lymphocytes, or micronuclei in bronchial tissue. Of these, only AHH induction and possibly micronuclei may be part of a direct pathway from exposure to disease, but any of these markers might be more predictive of lung cancer than smoking history.

One limitation of some biomarkers is that they may represent only recent exposure effects rather than cumulative effects over time. For example, cotinine is a measure of smoking over the previous several days. Sister chromatid exchange in peripheral lymphocytes of smokers measures the effects of smoking over a period of months. Biomarkers that reflect only recent exposures will not be good predictors of chronic diseases produced by historical exposures.

Here we will use the term "biomarker" with the understanding that the marker may be interpreted as either a marker of exposure or an early sign of disease and that it may or may not lie on a causal pathway between exposure and disease (*i.e.*, be a true intermediate variable). Our discussion will be limited to the scenario in which it is assumed that a given exposure causes both the biomarker and disease, and the question is whether the biomarker is a more useful predictor of disease than external exposure [for a general discussion of biomarkers, see Hulka *et al.* (1)]. The focus will be on observational studies, and our treatment builds on prior discussions by Schatzkin *et al.* (2), Freedman *et al.* (3), and Prentice (4).

Received 11/2/92; revised 1/4/93; accepted 1/4/93.
[1] This research was performed in part under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract no. W-7405-ENG-48.
[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: AHH, aryl hydrocarbon hydroxylase; SCE, sister chromatid exchange; CA, chromosomal aberrations; SMR, standardized mortality ratio.

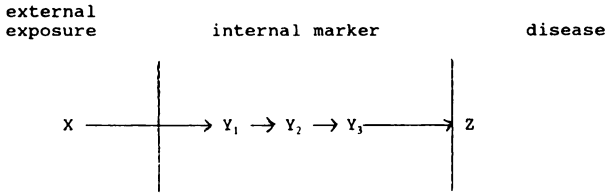

Fig. 1. Y_1 is an intermediate variable.

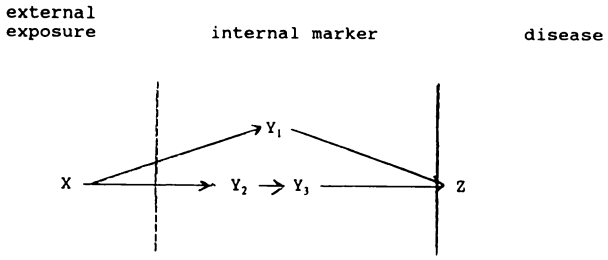

Fig. 2. Y_1 is an intermediate variable but X acts through another pathway as well.

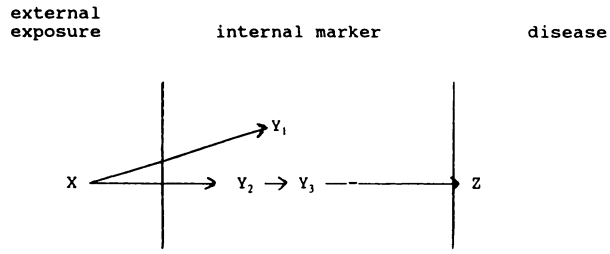

Fig. 3. Y_1 is an ancillary variable; X acts through another pathway.

Causal Pathways and Assessing the Predictive Values of Biomarkers

Figs. 1-4 illustrate some of the many possibilities in the relationship between exposure (X), the marker (Y_1), and disease (Z). In Figs. 1–4 it is assumed that there is a causal relation between exposure X and both the marker Y_1 and the disease Z. Y_2 and Y_3 represent intermediate but unknown and unmeasured steps on the direct pathway between exposure and disease. In Figs. 1–4 the variables may be either dichotomous (present or absent) or continuous. Disease does not automatically occur given the presence of external exposures or internal markers in any of the figures; *i.e.*, causes as shown here are not sufficient, nor are they necessary (5).

Figs. 1–4 are schematic only. They omit scenarios in which other exposures also cause the marker to occur, in which two exposures interact to cause a marker, or in which two markers interact to cause disease. More importantly, Figs. 1–4 omit key biological considerations and crucial details regarding the timing of exposures and markers in relation to disease. They do not specify if the exposure X is a one-time or chronic exposure, nor do they specify if the marker Y_1 is a reflection of recent exposure or past exposures. In Fig. 1, Y_1 is an intermediate variable on the causal pathway between exposure and disease; *e.g.*, exposure to the bladder carcinogen 4-aminobiphenyl may lead to abnormal cytology in exfoliated bladder cells, which might be viewed as an intermediate variable predictive of a clinical bladder tumor. When Y_1 is a true intermediate variable, it may be assumed that Y_1 will be a better predictor of disease than X. For dichotomous variables this assumption means that $\Pr(Z = 1 \mid Y_1 = 1) > \Pr(Z = 1 \mid X = 1)$, while for continuous variables (X, Y_1, and Z continuous), Y_1 is likely to be more highly correlated with Z than with X.

In Fig. 2, Y_1 is an intermediate variable on the causal pathway, but there is a second independent pathway by which X can also lead to Z. Y_1 might be the presence of fibrosis after exposure to asbestos, which might increase the risk of lung cancer independently of the level of external dose. However, asbestos without fibrosis may also lead to lung cancer. In this case, it is not clear whether the level of external dose or the presence of fibrosis would be likely to be a better predictor. Conventional statistical techniques (*e.g.*, stratification) may not adequate to estimate separately the effects associated with each pathway (6).

In Fig. 3 Y_1 is ancillary, in that it is not on the pathway between X and Z. Furthermore, we assume that Y_1 has no independent association with Y_2 (or Y_3) or Z. Although there will be a weak association between Y_1 and Y_2 simply because X causes both Y_1 and Y_2, one might still expect X to be a better predictor of Z than Y_1; *e.g.*, asbestos might cause pleural plaques, but pleural plaques may be unrelated to lung cancer. The number of pleural plaques would probably be less predictive of lung cancer than the level of asbestos exposure (however, a contrary scenario could be imagined if pleural plaques were a more accurate measure of true asbestos exposure than the actually measured asbestos exposure).

In Fig. 4, Y_1 may be a better predictor of Z than X because Y_1 is associated with Y_2 (independently of X). An example would be DNA adducts (Y_1) measured in the placenta of pregnant smokers, which have been shown to be better predictors of low birth weight than smoking history (X), even though they might not be on any causal pathway between smoking and low birth weight (here Y_2 is unknown) (7).

Data on exposure, markers, and disease from observational studies generally will not enable us to determine which (if any) of Figs. 1–4 are applicable (although it may provide clues). However, it is possible and worthwhile to address the empirical question of whether the external exposure or the biomarker is a better predictor of disease. For example, if the marker is more predictive of disease than exposure, this may have implications minimally for directing future research toward the marker or maximally for intervention to cease exposure or for treatment to prevent future disease. Under the assumption that exposure X precedes and causes marker Y_1 and disease Z, this question can be answered statistically by a simple test of whether the marker is more highly correlated or associated with the outcome than external exposure. This is a qualitative test for relative predictive value, given the uncertainty of underlying mechanisms, and can be considered a test of validity of the marker only if we limit our definition of "validity" to a better "predictor" rather than "on a causal pathway." Ignorance of the true causal pathway means that even if we know Y_1 is a better predictor of disease than X, this does not necessarily imply that intervention on Y_1 will have a preventive effect on disease.

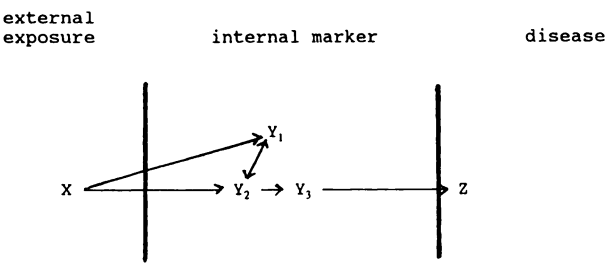

Fig. 4. Y_1 is an ancillary variable but associated with a variable on the causal pathway.

A second, conditional test for the predictive ability of the biomarker is whether, given the same level of exposure, those with the biomarker present (or with higher levels of the biomarker) are more likely to develop disease [*i.e.*, $\Pr(Z = 1 \mid Y_1 = 1, X = x_i) > \Pr(Z = 1 \mid Y_1 = 0, X = x_i]$. This test considers whether Y_1 predicts Z after taking X into account (note, however, that even if Y_1 steals all the predictive value of X, one may not conclude that X does not cause Z). The conditional test is related to some tests proposed in the literature for validity of surrogate endpoints (2–4). Aside from our ignorance of underlying mechanisms, one problem with these seemingly simple tests is that they do not consider the timing of the exposure and the marker or the persistence of the marker. Another issue not considered is measurement error, which typically affects the external exposure (often estimated historically) more than the marker (although markers themselves exhibit large intrapersonal variability). This may attenuate the observed exposure-disease relationship more than the marker-disease relationship.

Few data are available on the predictive value of most biomarkers for diseases with onsets soon after exposures. There is even less information regarding the predictive value of markers for chronic disease with long induction periods between exposure and onset. By way of example, for the remainder of this article we focus on two markers (cytogenetic changes and lung fibrosis) which may be related to future cancer.

Carcinogens, Cytogenetic Changes, and Cancer

Background: Chromosomal Aberrations, Sister Chromatid Exchange, and Micronuclei. The most common cytogenetic outcomes used in epidemiological studies to date are CAs, micronuclei, and sister chromatic exchange (1). Cytogenetic effects measured with these outcomes generally do not persist, so that these outcomes will reflect recent exposures. This feature is one of the main limitations in using cytogenetics in epidemiological studies to predict chronic disease following an exposure.

One exception to this short persistence is stable CAs. While unstable CAs (*e.g.*, dicentrics and deletions) cause cell death at cell division, and their half-life in lymphocytes is only a few years (8), stable aberrations such as balanced translocations do not cause cell death and may persist throughout cell division. Unstable CAs have been the focus of most studies to date; until recently the analysis of stable CAs required the use of chromosome banding which is expensive and time consuming. Recently a new procedure called chromosome painting was developed (9). This is a faster and less expensive technique for analyzing balanced translocations, using chromosome-specific composite DNA probes and staining with immunohistochemical methods.

Translocations may prove useful as a biodosimeter decades after exposure (10), when the unstable aberrations would not be elevated. Painting may also be useful for quantifying chronic low-level exposure because of the (theoretical) ability of stable aberrations to accumulate as a function of both duration and intensity of exposure. However, to date this new technique has not been used extensively and will not be the focus of discussion here.

Case-Control Approaches to Measuring Predictive Value. Regarding the value of the cytogenetic outcomes described above for predicting cancer, two obvious problems are: (*a*) their measurement in blood cells which are not the target organ of many cancers; and (*b*) the limited persistence (instability) of some types of cytogenetic events and the problem of temporal sequence.

These factors limit the possibility of using a case-control design to evaluate the predictive value of cytogenetic outcomes. Perhaps the most damaging is the problem of temporal sequence; the cytogenetic changes must precede disease onset but occur after exposure, but this sequence often cannot be determined. The use of a case-control design to evaluate the relative predictive power of exposure data *versus* cytogenetic outcome will rely on a comparison of cytogenetic outcomes in cancer patients compared to controls, both with known data on the history of exposure to a carcinogen. An elevation in cytogenetic outcomes, measured at time of diagnosis (characteristic of case-control studies), may be an effect of the cancer itself rather than evidence that those with elevated markers were more likely to develop cancer. Two recent case-control studies which have found elevated SCEs in pretreatment cancer patients concluded that the elevations were the product of the disease rather than the cause (11, 12). Furthermore, elevated cytogenetic findings will generally reflect recent exposures, while recent exposures probably would not be responsible for the development of cancer which generally requires a latency of 10–20 years (in the future, analysis of stable translocations may make some retrospective analyses feasible).

These basic problems with a retrospective approach are so great as to make a case-control design currently of little use in resolving the issue of relative predictive power.

Prospective Cohort Approaches to Measuring Predictive Value. More promising in theory are studies which take a defined group exposed to a potential carcinogen with known levels of exposure, measure the cytogenetic outcomes shortly after or during exposure, and follow the group over time until cancer develops. Cancer incidence in the exposed is then compared to cancer incidence among a nonexposed comparison group. Ideally, the cytogenetic outcomes would also be measured in the nonexposed group. A case-cohort or nested case-control approach may be used when the relevant biological samples may be stored without deterioration.

In practice, such an ideal study has not occurred, due to the enormous expense and logistic difficulties involved. Two populations which might potentially be studied are survivors of atomic bombs and patients irradiated for treatment with ankylosing spondilitis. Both these groups have been studied for chromosomal aberrations and separately for cancer incidence, but the two outcomes have not yet been studied together (8, 13).

Another possible cohort which might be studied are cancer patients treated with known doses of radiation or chemotherapy and tested shortly thereafter for cytogenetic outcomes. These patients are at high risk of a second tumor.

A number of investigators have studied cytogenetic outcomes in such patients (14). No follow-up study of cancer incidence among such patients after cytogenetic testing has been reported.

There has been one large prospective study of cytogenetic outcomes and subsequent cancer which has been reported in the literature, the Nordic cohort study of 3000 individuals whose lymphocytes were tested for SCEs, CAs, and micronuclei between 1970 and 1985, with follow-up for cancer incidence through 1985 (15). Unfortunately, this study is not applicable to the question at hand because of the lack of any defined exposure to a carcinogen (the premise underlying this study is that cytogenetic outcomes may serve as markers of cancer susceptibility, even without knowledge of specific carcinogenic exposures).

Can Animal Studies Provide an Answer? Questions about relative predictive power can perhaps be answered by animal studies, although one is then faced with the usual problem of the applicability of animal data to humans. Rodent studies of ethylene oxide provide a model of how this might be done. Ethylene oxide is a known producer of SCEs, CAs, and micronuclei in humans and rodents and a cause of leukemia and brain cancer in rats. It would be possible to expose rats to a dose of ethylene oxide in an inhalation chamber (*e.g.*, 100 ppm either continuously over a lifetime or a higher shorter exposure), take blood for measurement of cytogenetic outcomes, and then determine which animals develop cancer. Cells from bone marrow and spleen might also be obtained at final sacrifice and still reflect cytogenetic damage if dosing had been continual.

Asbestos, Fibrosis, and Lung Cancer

Background. It is well known that high levels of exposure to asbestos may result in fibrotic lung disease and that there is a clear dose-response pattern to this relationship (16). It is also well known that cohorts of workers exposed to asbestos experience an elevated rate ratio for lung cancer, approximately 1.5–5.0 compared to nonexposed referents (17). Finally, there are also data showing that cohorts of asbestotics have even higher rate ratios for lung cancer ranging from 3.5 to 9.1 (16, 18–22). One issue which remains unclear from these data is whether the high lung cancer rate ratios for asbestotics are a reflection simply of higher doses of asbestos (a lung carcinogen), or whether asbestosis itself is a risk factor for lung cancer independent of level of asbestos exposure.

Animal and Human Data. Wagner *et al.* (23) conducted asbestos inhalation studies in which rats (n = 157) which did not develop asbestosis had a lung cancer incidence (4%) similar to the background incidence in nonexposed animals, while 44 rats with the same exposure which did develop asbestosis had a significantly higher lung cancer incidence (11%). Davis *et al.* (24) noted that bronchoalveolar hyperplasia developed in areas of interstitial fibrosis in rats, which in turn led to subsequent neoplasia. Kuschner (25) has reviewed the animal data and concluded that fibers must induce fibrosis in order to cause cancer. Browne (26) concluded a 1986 review of the subject by stating that "epidemiology may be too blunt an instrument to bring much further enlightenment" but that data from experimental pathology suggested that neoplastic change may appear only in the wake of inflammation and subsequent fibrosis and adenosis." This conclusion would imply either that (*a*) asbestos exposure acts via a direct pathway but must pass a threshold prior to causing cancer, with fibrosis occurring via a different pathway at a lower threshold; or (*b*) asbestosis is a necessary intermediate step on the pathway from exposure to disease.

In humans, Liddell and McDonald (16) have studied asbestos-exposed workers in Canada who had radiographs taken at work. Within this cohort, 118 died of lung cancer. Those with abnormal chest radiographs indicating asbestosis (n = 1472) had a significantly elevated SMR of 3.24 compared to those with normal radiographs (n = 3087). Comparing both groups to the general population, the respective SMRs were 3.50 and 1.08. The data presented in the report do not permit one to disentangle the effects of cumulative dose and asbestosis, although they do suggest that the lung cancer risk in this cohort was primarily restricted to those with asbestosis.

Hughes and Weill (21) have described the lung cancer mortality of 646 men producing asbestos cement. In analyses restricted to those with long duration of exposure (>21.5 years), those with no radiographic abnormalities (n = 211) had a respiratory cancer SMR of 1.03 (6 observed), while 77 men with small opacities ≥ 1/0 (indicating fibrosis) had a significantly elevated SMR of 4.32 (9 observed). The estimated cumulative asbestos exposure and duration of exposure of the two groups was comparable. Among those with no abnormalities on X-ray, (n = 420), there was no apparent dose-response for estimated cumulative exposure and lung cancer. Survival analyses within the exposed cohort indicated that after adjustment for age and smoking, neither estimated cumulative nor average asbestos exposure was a significant predictor of lung cancer, while on the other hand X-ray abnormality was a significant predictor.

Other relevant data come from a study by Slius-Cremer and Bezuidenhout (27, 28), who conducted a case-control study among 427 autopsied decedents (35 lung cancers) in a cohort of asbestos workers. Only 37% of the decedents had autopsies, and there are possible biases in these data typical of autopsy studies. In these data, duration of exposure was a better predictor of lung cancer than estimated cumulative asbestos exposure. After entering age and smoking into the model, both duration of exposure and the presence of asbestosis were each (singly) highly predictive of lung cancer, without one being clearly more important than the other. In other analyses matched by duration of exposure, asbestosis continued to be a significant predictor of lung cancer.

These human data add support to the view that the presence of asbestosis is as good as or a better predictor of lung cancer risk than is cumulative exposure.

Discussion

While no general conclusion is possible, it seems likely that observational epidemiology will often be too imprecise a tool to sort out the effects of exposure and marker on the disease outcome, particularly for chronic diseases, without excellent quantitative and temporal data on both exposure and on some internal marker which persists over time. Animal studies are likely to be more useful, although not generalizable to humans. For example, the role of the induction of the cytochrome P450 enzyme system by dioxin for the subsequent development of cancer can possibly be investigated in rats. After a single level of dose, induction might be measured via urinary metabolites of caffeine, while liver tumors can be assessed at sacrifice.

There are instances in which adequate human data has been (or could be) collected. Intervention studies and clini-

cal trials, in which exposure is a treatment, may provide a better setting than observational studies to evaluate the question. However, adequate data from observational epidemiological studies are sometimes available. An example is the importance of a decrement in lung function in predicting mortality among smokers. Curb *et al.* (29) have recently shown that among never-smokers lung function is not predictive of overall mortality, while among smokers it may be a better predictor than pack-years of smoking (although a complete data analysis on this point is not provided). Another example is the apparent superior predictive power compared to traditional risk factors of left ventricular hypertrophy in predicting heart disease (30). Such hypertrophy may be an intermediate variable between elevated blood pressure and heart disease.

These issues will be of increasing importance as more data on markers or intermediate outcomes become available. Validated markers which can predict disease could become useful screening tools leading to treatment if the disease is treatable or could lead to prevention via cessation of exposure if the marker is on a direct pathway between exposure and disease.

This discussion has been focused on the relative predictive value of biomarkers for chronic disease, compared to an external exposure. We are not suggesting that biomarkers in general have limited use for epidemiologists, but rather that caution must be exercised in the interpretation of data on exposure *versus* markers in considering chronic diseases, especially when we are ignorant of causal pathways and temporal sequences. The predictive value of exposure *versus* biomarkers for acute diseases, without long induction periods, will probably be easier to assess epidemiologically. Other markers are useful in determining susceptibility to disease (*e.g.*, AHH inducibility) or in identifying early evidence of disease (dysplasia of cervical cells), irrespective of any specific exposure.

Acknowledgments

Drs. Paul Schulte, Richard Hornung, Arthur Schatzkin, Lawrence Freedman, and Janet Hughes kindly reviewed the manuscript.

References

1. Hulka, B., Wilcosky, T., and Griffith, J. (eds). Biological Markers in Epidemiology. New York: Oxford, 1990.

2. Schatzkin, A., Freedman, L., Shiffman, M., *et al.* Validation of intermediate endpoints in cancer research. J. Natl. Cancer Inst., *82:* 1746–1752, 1990.

3. Freedman, L., Graubard, B., and Schatzkin, A. Statistical validation of intermediate endpoints for chronic diseases. Stat. Med., *11:* 167–178, 1992.

4. Prentice, R. Surrogate endpoints in clinical trials: definition and operational criteria. Stat. Med., *8:* 431–440, 1989.

5. Rothman, K. Modern Epidemiology. Boston, MA: Little Brown, 1986.

6. Robins, J. and Greenland, S. The nonidentifiability of direct and indirect effects. Epidemiology, *3:* 143–155, 1992.

7. Everson, R., Randerath, E., Santella, R., *et al.* Quantitative associations between DNA damage in human placenta and maternal smoking and birth weight. J. Natl. Cancer Inst., *8:* 567–576, 1988.

8. Buckton, K., Hamilton, G., Payton, L., *et al.* Chromosomal aberrations in irradiated ankylosing spondilitis patients *In:* H. Evans and B. Lloyd B. (eds.), Mutagen Induced Chromosome Damage in Man, pp. 142–150. Edinborough: Edinborough University Press, 1978.

9. Pinkel, D., Landegent, J., Collins, C., *et al.* Fluorescence *in situ* hybridization with human chromosome-specific libraries: detecton of trisomy 21 and translocations of chromosome 4. Proc. Natl. Acad. Sci. USA, *23:* 9138–9142, 1988.

10. Straume, T., Lucas, J., Bigbee, W., *et al.* Biodosimetry for a radiation worker using multiple assays. Health Phys., *62:* 122–130, 1992.

11. Adhvaryu, S., Dave, B., Trivedi, A., *et al.* Heteromorphism of C-band positive chromosomal regions in CML patients. Cancer Genet. Cytogenet., *27:* 33–38, 1987.

12. Yokota, K., Udea, K., Ohama, K., *et al.* Increased spontaneous and mitogen C-induced SCE's in patients with cancer of the cervic uteri. Cancer Genet. Cytogenet., *43:* 79–87, 1989.

13. Bloom, A., Nakagome, Y., Awa, A., *et al.* Chromosome aberrations and malignant disease among A-Bomb survivors. Am. J. Public Health, *4:* 641–644, 1970.

14. Tucker, J., Wyrobek, A., Ashworth, L., *et al.* Inductions, accumulation, and persistence of SCEs in women with breast cancer receiving cyclosphosphamide, adriamycin, and 5-fluorouracil chemotherapy. Cancer Res., *50:* 4951–4956, 1990.

15. Brogger, A., Hagmar, L., Hansteen, I., *et al.* An inter-Nordic prospective study on cytogenetic endpoints and cancer risk. Cancer Genet. Cytogenet., *45:* 85–92, 1990.

16. Liddell, F., and McDonald, J. Radiological findings as predictors of mortality in Québec asbestos workers. Br. J. Ind. Med., *3:* 257–267, 1980.

17. IARC. Supplement 7. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs 1 to 42. Lyon, France: International Agency for Research on Cancer, 1987.

18. Berry, G. Mortality of workers certified by pneumoconiosis medical panels as having asbestosis. Br. J. Ind. Med., *38:* 130–137, 1981.

19. Finkelstein, M., Kusiak, R., and Suranyi, G. Mortality among workers receiving compensation for asbestosis in Ontario. J. Can. Med. Assoc., *3:* 259–262, 1981.

20. Cookson, W., Musk, A., Glancy, J., *et al.* Compensation, radiographic changes, and survival in applicants for asbestosis compensation. Br. J. Ind. Med., *42:* 461–468, 1985.

21. Coutts, I., Gilson, J., Kerr, I., *et al.* Mortality in cases of asbestosis diagnosed by a pneumoconiosis medical panel. Thorax, *42:* 111–116, 1987.

22. Hughes, J. and Weill, H. Asbestosis as a precursor of asbestos-related cancer: results of a prospective study. Br. J. Ind. Med., *48:* 229–233, 1991.

23. Wagner, J., Berry, G., and Timbrell, V. Mesothelioma in rats after inoculation with asbestos and other materials. Br. J. Cancer, *28:* 173–185, 1973.

24. Davis, J., Bolton, R., Brown, D., *et al.* Experimental lesions in rats corresponding to advanced human asbestosis. Exp. Mol. Pathol., *44:* 207–221, 1986.

25. Kuschner, M. The effects of MMMF on animal systems: some reflections on their pathogenesis. Ann. Occup. Hyg., *48:* 791–797, 1987.

26. Browne, K. Is asbestos or asbestosis the cause of the increased risk of lung cancer in asbestos workers? Br. J. Ind. Med., *43:* 145–149, 1986.

27. Slius-Cremer, G., and Bezuidenhout, B. Relations between asbestosis and bronchial cancer in amphibole asbestos miners. Br. J. Ind. Med., *46:* 537–540, 1989.

28. Slius-Cremer, G., and Bezuidenhout, B. Relations between asbestosis and bronchial cancer in amphibole asbestos miners (letter). Br. J. Ind. Med., *47:* 215–216, 1990.

29. Curb, J., Marcus, E., Reed, D., *et al.* Smoking, pulmonary function, and mortality. Ann. Epidemiol., *1:* 25–32, 1990.

30. Levy, D., Garrison, R., Savage, D., *et al.* Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N. Engl. J. Med., *22:* 1561–1566, 1990.