Pesticides are widely dispersed in the environment and exposure to them is almost unavoidable, mainly through the food chain. During the peak period of its use, DDT was so ubiquitous that it could be detected in ice core samples taken in the Antarctic, even though it had never been used on that continent. Pesticides have been one of the most intensely studied of possible carcinogens in the environment. As with other environmental exposures, epidemiological research into the health effects of chronic pesticide exposure is subject to methodological challenges, and our understanding of the relationship between exposure and health remains limited. This article describes some of the challenges facing environmental epidemiology, and some of the recent developments in molecular epidemiology that may assist these challenges. To assist the reader, a glossary of terms is provided toward the end of the article.

SOME CURRENT CHALLENGES IN ENVIRONMENTAL EPIDEMIOLOGY

One of the challenges facing environmental epidemiology is getting accurate information on pesticide exposure. This challenge is exacerbated by the initiation and latency periods that are usually associated with cancer, which means that there may be lag periods of more than 10 years between an exposure and a particular outcome of that exposure. Any prospective study that looks at the health effects of a current exposure will necessarily involve long follow-up periods, even if the current exposure can be accurately determined, and any retrospective study will need to determine the effects of exposures many years in the past. Where data on exposure is dependent on the recall of those individuals subject to the exposure, measurement errors—in the form of ‘recall bias’—are also likely.

Numerous surveys have been taken of pesticide levels in air, water, and food. However, because individuals vary in their behaviour—in relation to air, water, and food—it is difficult to extrapolate survey data to estimate individual exposure levels in a community setting. Also, exposures in air, water, and food are likely to be low, particularly since the 1960s when restrictions on the use of ‘persistent’ pesticides (that is, pesticides that persist in the environment) became widespread in western societies. At the levels likely to be faced in these communities, epidemiological studies would need to demonstrate large increases in carcinogenic risk to confidently identify an association between a particular pesticide and a form of cancer.

To overcome these difficulties, researchers have frequently turned to occupational settings to explore the relationship between exposure and health, since occupational exposures are likely to be higher and more predictable. However, even in an occupational setting, pesticide
exposure tends to be difficult to assess because the users of pesticides rarely have standardised work practices.

Information on individual exposures for pesticide users is also often limited. To overcome this, researchers have often turned to simple occupational categorisation to define exposure groups. However, to be most effective, such an approach requires homogeneity in the exposures likely to be experienced by individuals identified in each category. Where, for example, categories such as ‘farm hand’ are identified from census or other routinely-collected documents, heterogeneity of exposure lessens the ability of these studies to detect true associations.

One frequently-used method of getting more accurate information on individual exposure in occupational settings is biological monitoring. Biological sampling for persistent pesticides such as the organochlorines, or for contaminant pesticides such as dioxins, can give a meaningful picture of total exposure over a number of years. However, metabolism and excretion of modern pesticides is rapid, and results of biological monitoring may only reflect recent exposure in the last few hours or days.

Some of the issues around the assessment of pesticide exposure were examined by a review of studies of the possible effects of Agent Orange, which was used by American military personnel during the Vietnam War. Until 1992, the assessment of exposure relied on categorisation of individuals into occupational groups that were thought likely to have worked with Agent Orange. Sometimes this assessment was supplemented by an individual’s own estimate of the exposure. However, in 1992 the United States Air Force completed a study examining the relationship between individual serum TCDD (the dioxin contaminant of Agent Orange) and verified reproductive outcomes. TCDD levels correlated poorly with both self-reported exposure and exposure indices developed from military records, which confirms the limitations of research dependant on these surrogate measures and the need for a degree of scepticism when interpreting the findings.

Another challenge for epidemiological studies exploring the health impact of pesticides is the ‘healthy worker effect’, where relatively healthy individuals tend to be more likely to gain employment and remain employed. This effect has the potential to bias studies towards finding lower mortality rates in an occupational cohort, when compared with the general community, and thus mask true increases in mortality. When studying the impact of pesticides, the ‘healthy worker effect’ may be complicated by the unique dietary and lifestyle factors associated with residing and working on a farm, which is associated with mortality and cancer rates below those of the broader community.

Finally, a range of other factors may confound the relationship between pesticide exposure and cancer mortality. These possibly include smoking, carcinogenic animal viruses, and the lymphoproliferative effect of prolonged antigenic stimulus. Our understanding of these risk factors is currently limited and inconsistent.

**SOME RECENT DEVELOPMENTS IN MOLECULAR EPIDEMIOLOGY**

Recent developments in the field of molecular epidemiology may assist the challenges facing environmental epidemiology, by providing better information on exposure and earlier information on outcomes, and by identifying members of the community who may be most sensitive to exposures to pesticide. A framework for applying these recent developments is outlined in Figure 1, which is based on the work of Fowle and Sexton.²

**Biomarkers of exposure**

Accurate assessment of exposure to potential environmental carcinogens will contribute to the accuracy of studies, and will reduce the number of subjects required to identify possible health effects. As mentioned above, chemicals that persist in the environment can already be measured directly in body tissues (sometimes at considerable expense). However, direct measurement of pesticides that are rapidly metabolised, such as organophosphates, is less useful; and the surrogate measures currently used, for example, serum cholinesterase levels, only provide a crude indication of exposure.

These problems may be partly overcome if other exposure-specific patterns of physiological or chromosomal effect could be identified. For example, xenobiotic-specific DNA adduct formation (complexes that form when a chemical binds to a biological molecule such as DNA) has been demonstrated to correlate with exposure to a number of toxic compounds including polycyclic aromatic hydrocarbons and nitrosamines. While there is still more work to be done in this area, discovery of exposure-specific DNA adducts will significantly improve our ability to accurately estimate exposure to various environmental carcinogens. Unfortunately, adducts linked to specific pesticides have not yet been identified.

**Measures of biological effect**

Most prospective studies of environmental exposures have relied on crude and relatively rare measures of biological effect such as mortality or cancer. One of the early characteristics of carcinogenesis is genetic damage. By using such damage as an intermediate indicator of outcome, molecular epidemiology may allow shorter follow-up periods and smaller study sizes. Indicators that have so far been linked to both outcome and pesticide exposure include chromosomal aberrations, sister
chromatid exchange, and micronuclei in peripheral lymphocytes.

**Exposure-susceptibility interactions**

One of the most exciting new approaches allows a better understanding of individual variation and to identify populations at risk. Researchers already control for a number of key causes of variability: for example age, ethnicity, and gender. Molecular epidemiology may also soon allow for improved assessment of other non-genetic factors such as smoking, for example by the use of PAH adducts as markers of past exposure.

A number of genetic susceptibility factors are also worthy of incorporation into studies of environmental carcinogenesis, in particular the relatively common genetic polymorphisms that determine the metabolic fate of pesticides. Metabolism of most pesticides is undertaken in a two stage hepatic process and/or by serum paraoxonase. Polymorphisms of the enzymes used in these processes are common. Subjects with less effective metabolic phenotypes may be expected to face a greater internal dose following exposure to a particular pesticide and thus be more susceptible to any adverse effect. Identifying susceptible individuals and studying them separately increases the chance of an epidemiological study identifying a true association.

Such molecular approaches have already been used in a number of studies exploring the carcinogenic potential of pesticides. Typically, these studies have been small and have explored the relationship of quantified exposure to intermediate indicators. More recently they have also examined variations in these relationships between subjects with different metabolic phenotypes.

**CONCLUSION**

While the findings of these early studies in molecular epidemiology have been inconsistent they suggest that, as we become more familiar with the techniques, we will be better equipped to understand the role pesticides and other environmental exposures play in carcinogenesis.

**REFERENCES**