PURDUE PESTICIDE PROGRAMS

Purdue University Cooperative Extension Service

PESTICIDES AND EPIDEMIOLOGY

Unraveling Disease Patterns

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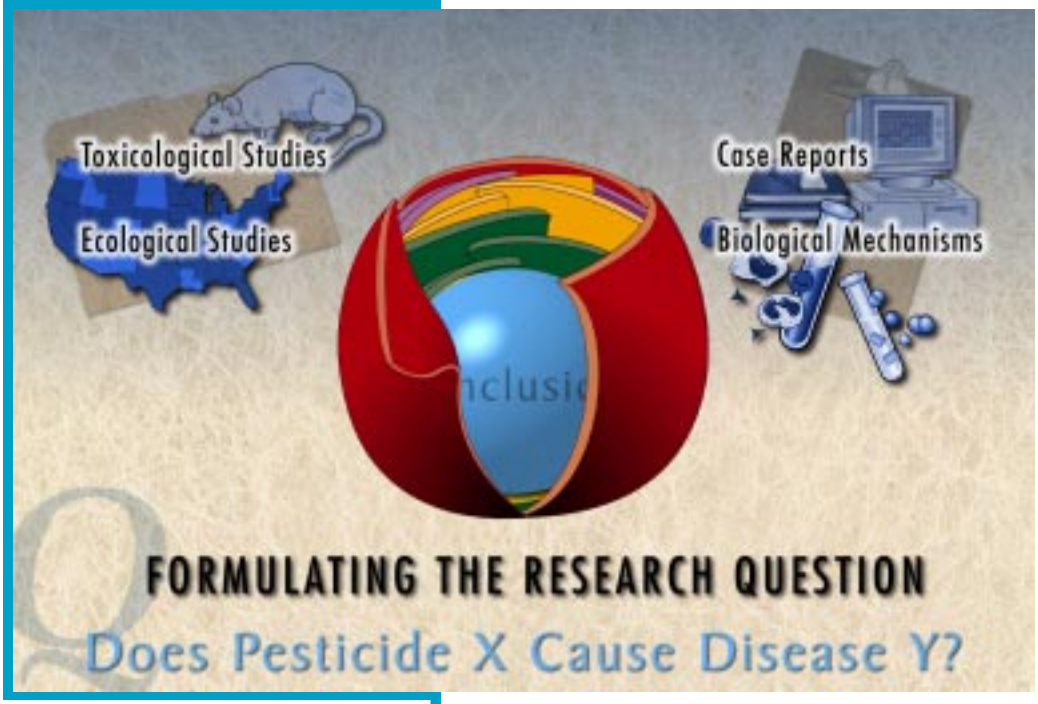
The Science of Epidemiology

Epidemiology is the study of the distribution and causes of disease in human populations. Epidemiologists focus on determining which factors cause disease and which factors protect against disease. Although modern epidemiology is considered a relatively young science, its basic concepts have aided society for hundreds of years in understanding causes of diseases such as cholera and lung cancer. By identifying causes of disease and populations which may be at highest risk, steps can be taken to minimize occurrence.

Potential human health effects from today's pesticides are first investigated using controlled experiments on laboratory animals. These facilitate predictions as to how pesticides may affect human health; data from such studies are critical in setting acceptable human exposure limits. Nevertheless, it is important to continue to investigate potential human health risks, directly, and epidemiology presents that opportunity. Epidemiological studies may examine whether rate of disease in an exposed population is different (higher or lower) than in a similar, unexposed population.

Epidemiological studies rest on one key assumption: In the absence of exposure, two human study populations will exhibit similar or identical rates of disease. Therefore, any variance in an exposed population is attributable to exposure.



Epidemiology and Pesticides

Pesticides are designed and developed to be toxic to specific living organisms; therefore, there is logical concern for the potential of these chemicals to adversely affect exposed human populations.

Epidemiological investigations increasingly address pesticides and their potential association with human disease. This increased concern for human toxicity potential addresses various levels (high, medium, low, absent) through various routes of exposure (food, air, water, soil).

The process of identifying causes of disease within pesticide-exposed populations is complex, primarily because pesticides are merely representative of many environmental exposures that people may encounter. Therefore, to say that a pesticide is associated with increased adverse health effects—cancer, respiratory problems, immune disorders, birth defects—requires

- the determination of a positive association between pesticide exposure and a specific disease, and
- that other known causes be ruled out.

For instance, farmers are not exposed *only* to pesticides, but also to other potential risk factors such as fertilizers, nitrates, fuels and engine exhausts, solvents, organic and inorganic dusts, electromagnetic radiation, ultraviolet radiation, and animal pathogens. Behavioral, dietary, and genetic factors may impact their risk of disease, as well.

Understanding a few basic epidemiological concepts and methods helps to comprehend epidemiological findings cited in scientific journals, media accounts, public advocacy group releases, and government documents. The intent of *Pesticides and Epidemiology* is to provide an overview of epidemiological methods and conclusions drawn from such studies.

Person, Place, and Time

The main objectives of epidemiology are to

- *describe* the occurrence of disease, and
- *explain* the possible causes of disease by identifying and quantifying etiologic (risk) factors.

Describing a disease requires the gathering of information on the distribution of disease in human populations based on age, gender, race, and geographical area. When little is known about the cause and occurrence of a disease, epidemiologists often study disease patterns as the first step in generating a hypothesis.

Characteristics used to describe patterns of disease fit into three general categories:

- Person—who is getting the disease (studies based on personal characteristics such as age, gender, race, religion, occupation, and socioeconomic status)
- Place—where the disease is occurring, geographically
- Time—when the disease is happening (also includes studies on whether the disease rate is increasing, decreasing, or staying the same)

Risk Factors

Risk factors (e.g., personal characteristics and environmental factors) are known to influence the distribution of disease within a population. Differences in disease patterns between two test populations often can be explained by one or more of these risk factors: age; gender; ethnicity; and external exposure factors such as cigarette smoke, exercise, prescription drugs, fruit and vegetable consumption, occupation, and pesticides.

Epidemiological Studies Consider Exposure Relationships

Epidemiologists study acute and chronic diseases. Acute diseases develop soon after an exposure has occurred (e.g., food poisoning at a picnic) whereas chronic diseases develop over months or years after exposure. The time that elapses between initial exposure and disease detection is divided into two periods: *induction* and *latency.*

The induction period is the time between exposure and disease development. At the end of the induction period, disease is inevitable for affected members of a population. The induction period

may last hours (e.g., pathogen and food poisoning) or decades (e.g., smoking and lung cancer) depending on the exposure/disease relationship.

Latency refers to the time that lapses between disease development and detection. The total time lapse between exposure and disease diagnosis, therefore, is the sum of the induction and latency. The longer the time lapse of either period, the more difficulties arise in linking a specific exposure to a specific disease in an epidemiological study; imprecise memory of exposure, lack of records, and people moving away are a few of the reasons why. Studies of pesticides and chronic disease have to consider exposures that occurred decades before a disease diagnosis is made.

Sources of Information

Often overlooked aspects of epidemiological research are the quality and availability of data sources used by epidemiologists.

Disease Records

Personal Medical Records

Personal medical records are the most reliable source of information to confirm disease diagnosis, and date of diagnosis. However, gaining legal access to medical files requires concerted effort on the part of the investigator. A protocol must be in place to protect the confidentiality of the study participants and to make participation voluntary.

Hospital Discharge Data

Hospital discharge data include disease-related information such as the diagnosis, length of stay, and date of discharge; information regarding the patient's date of birth, race, gender, and area of residence is often available, as well. For certain diseases, however, diagnosis can be merely preliminary and should be verified by medical record review. Hospital discharge data can be useful to identify a cohort (a group of people who share common characteristics); but the identity of subjects is revealed only through informed patient consent, which can be cumbersome to acquire.

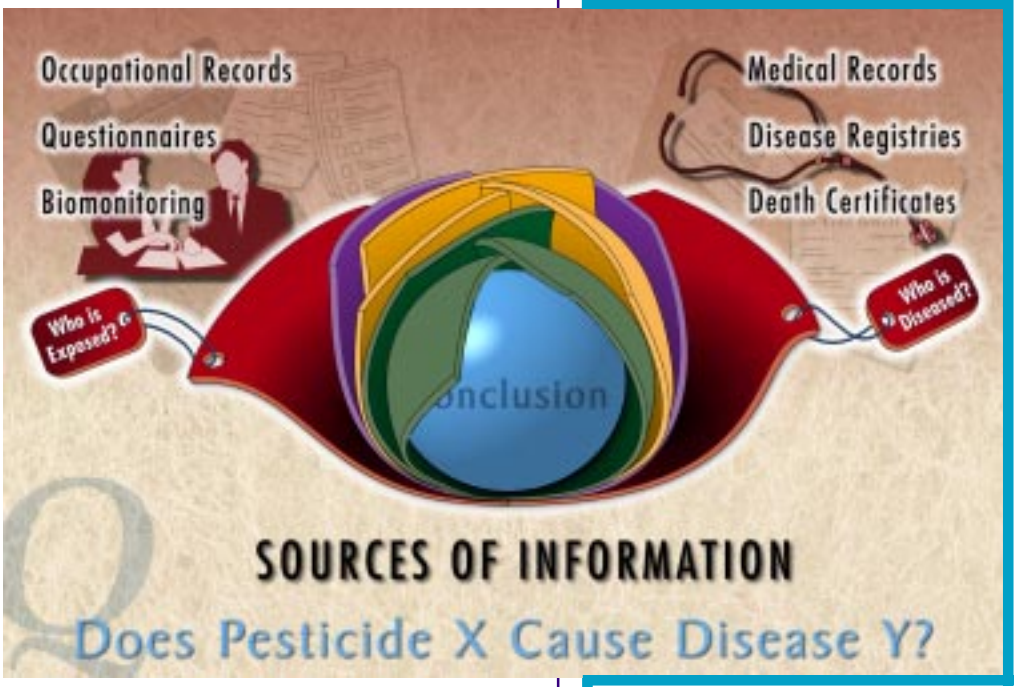
Disease Registries

Physicians are required by law to report certain diseases to public health authorities. In addition, there are ten population-based cancer registries in the United States (all supported and funded by the National Cancer Institute) which arrange to get diagnoses from various medical sources. In addition, the Center for Disease Control registry program interacts significantly with state cancer registries; and many states have their own state-funded disease registries.

While some registries compile information on the incidence of cancer, others may track the occurrence of birth defects (e.g., New York State Birth Defects Registry), communicable diseases such as AIDS, and kidney disease.

Death Certificates

A death certificate is the official and primary source of information on the cause of death. Underlying and supplemental causes are recorded on the death certificate, then coded and recorded by state vital statistics agencies. A death certificate also contains other information on the deceased: gender; age; race; marital status; occupation; education; place of residence; and the date, time, and place of death.



Birth Certificates

Birth certificates can be utilized in studies of etiologic (risk) factors that adversely affect reproduction; information on the newborn might include gender, birth weight, and documentation of any malformations. The ages of the parents and the number of previous pregnancies of the mother often are noted, as well.

Exposure Records

Under certain circumstances, epidemiologists can utilize actual measures of exposure as the basis for classifying the exposure status of study subjects. Exposure measurements in the workplace and in study subjects improve study validity and the overall interpretation of the study. For example, measurements of workplace surfaces frequently touched, or an individual's breathing zone, obviously are preferred bases for classifying workers' exposure.

Biomonitoring

Scientists have recently begun estimating exposures through biomonitoring: measuring the pesticide or one of its components in blood, urine, or fat. Such measurements form a good basis for determining who is exposed and, more importantly, the degree of exposure. Compounds that are fat soluble remain measurable in the body for years, while compounds that are fat insoluble can be measured in blood or urine for only a short time after exposure.

Occupational Records

Occupational records are another source of exposure data. The best occupational information comes from employment records maintained by individual companies.

Sometimes these records are detailed and well documented, containing a complete listing of an employee's work assignments, the beginning and ending dates of each, and even some personal exposure monitoring data. Frequent employment changes complicate the situation because the potential for exposure and the quality of employment records vary significantly among employers.

Other times, work records are quite poor or nonexistent. Epidemiological studies sometimes determine a decedent's occupation from the death certificate, although it usually states only the decedent's *last* job. Therefore, the data entered do not necessarily represent objective documentation: Results based on occupational data from death certificates are not always valid.

Questionnaires

Questionnaires may be distributed and completed in person, by telephone, or by mail. However, the resulting data must be interpreted cautiously because they are only as valid as the respondents' memory and state of mind at the time they complete the questionnaire. Undoubtedly, there are differences in circumstantial recall of various respondents; for example, diseased individuals

may be more thorough in filling out a questionnaire than those who are disease-free. It has been demonstrated that the care taken in questionnaire development and the circumstances of questionnaire administration affect the reliability of response interpretation. Data obtained from questionnaires can, however, provide valuable historical information.

Among the various types, person-to-person interviews provide the best response rate and quality of information, although they are more expensive to conduct and the results depend largely on the interviewer. Questionnaires completed by telephone or through the mail, while cheaper, often result in lower response rates—especially by mail. There are always questions about how much respondents differ from those who fail to respond.

Another issue affecting questionnaire response is the sensitivity of the questions asked. If questions deal with sensitive information, subjects may refuse to provide answers or may give answers that reflect their own bias. Sexual history, alcohol use, and drug abuse are examples of factors that might not be reported accurately on questionnaires unless special procedures are established to gain the respondents' trust.

EPIDEMIOLOGY AS AN OBSERVATIONAL SCIENCE

Epidemiological research often begins with a clearly formulated question and hypothesis:

- Question: Do commercial pesticide applicators who apply lawn care chemicals exhibit elevated rates of peripheral nerve damage?
- Hypothesis: Rates of peripheral nerve damage in pesticide applicators differ from rates in the general (unexposed) population.

The epidemiologist must design a study to evaluate the hypothesis in order to determine whether the population-at-risk —in this case, lawn care applicators—has an elevated rate of peripheral nerve damage. The term *population-at-risk* is misleading because it is not meant to imply that a group of people is actually experiencing an increased risk of disease. Rather, population-at-risk defines the population in which an exposure/disease relationship can be studied. For example, the population-at-risk is commercial lawn care applicators, the condition of interest is peripheral nerve damage, and the contributing risk factor is hypothesized to be pesticide exposure.

Researchers using traditional scientific methods control the circumstances of a study, that is, they determine who will be

exposed and who will not be exposed. Epidemiologists, however, cannot control exposure; ethical considerations render it impossible to expose populations to potentially toxic substances to observe whether disease develops. Hence, detection of the effects of pesticide exposure is predominantly an observational science.

While epidemiologists conduct observational studies under real world conditions which allow examination of a multitude of factors and interactions, often it is these real world conditions which cloud the disease/exposure relationship. Results from an observational study are not necessarily evidence of a causal exposure/disease relationship, but they do indicate that *exposure is associated with disease.*

Causation and association are two distinctly different concepts of affiliation between exposure and effect. *Causation* indicates that there is sufficient, strong evidence for scientific consensus. *Association* means that a relationship has been reported, but that the evidence is not strong enough to effect consensus.

One challenge in observational research is the identification, within the study groups, of any important characteristics *other than pesticide exposure* that might contribute to disease. Epidemiologists must rule out factors such as age, gender, and diet when attempting to link a disease with a specific exposure; without this accountability, association of the studied exposure cannot be verified. Epidemiologists must eliminate the role of all other factors in determining a valid association between test groups and incidence of disease.

Study Designs in Epidemiology

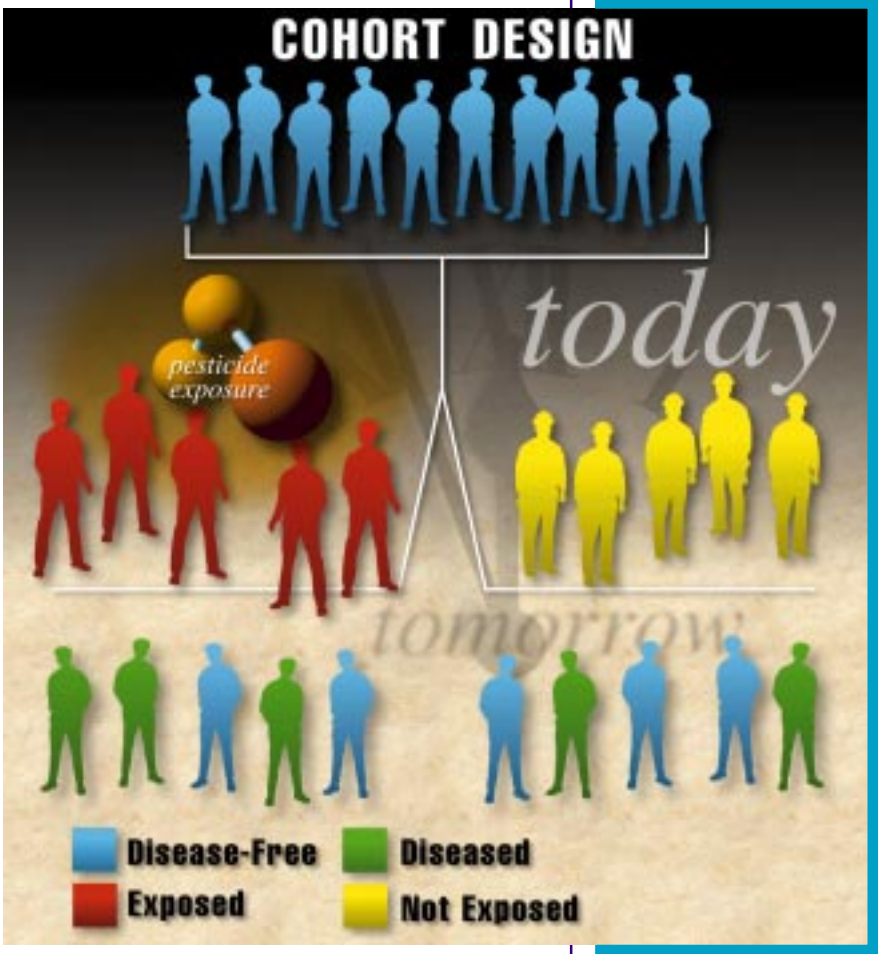
Two important study designs in epidemiology are *cohort* and *case-control.*

Cohort Design

Cohort studies begin with a group of people that share common characteristics—the cohort—and evaluate their health over an extended time period. A cohort might include Kansas wheat farmers, golf course superintendents, or certified pesticide applicators. The basic question addressed by a cohort design: *Is the exposed population more or less likely to develop disease than the unexposed population?*

A cohort design requires all subjects to be free of disease at the start of the study. All subjects are followed, over time, and their individual exposures and diseases documented. Ultimately, the cohort is separated, based on those who were exposed to a specific agent and those who were not. Disease occurrence is then analyzed to see if frequency varies between the exposed and unexposed groups.

Cohort designs can be either retrospective or prospective. The difference is simply the timing of data collection: whether the study proceeds from a previous point in time (retrospective) or from the current time, forward (prospective).



Retrospective Cohort Design

A retrospective cohort design focuses on a group exposed at some point in the past. The exposure point can be a documented historical event such as an explosion, a fire, a spill, or a date of employment. Once the retrospective date of initial exposure is determined, epidemiologists trace the study subjects' health status from that point in time to the end-of-study date. This type of study can be used on occupational groups such as employees of a pesticide manufacturing facility or those of a commercial pesticide application company.

Example: An epidemiologist might decide, today, to study the mortality of all current and former workers at a pesticide manufacturing plant who worked at least one year between 1950 and 1995. The workers at the manufacturing facility comprise the retrospective cohort. The epidemiologist reviews work histories and other available exposure information (e.g., air monitoring data at the work site) on individuals in the cohort to determine the exposure status of each worker.

The epidemiologist conducts extensive research into each worker's history from 1950 (or from the date of first employment, if after 1950) through 1995 to determine whether any of the workers died and, if so, their cause of death. Death rates of exposed workers in the cohort are compared with those of unexposed workers; local, state, and/or national mortality rates are compared, as well. Additionally, the epidemiologist may use rates of surrounding counties to ensure the comparison population is most similar to the worker population.

Prospective Cohort Design

A prospective cohort design focuses on a group of people from a current point in time through a future point in time. Example: the Agricultural Health Study being conducted in Iowa and North Carolina by the National Cancer Institute and the National Institute of Environmental Health Sciences. The study involves farmers, commercial pesticide applicators, and their families; each individual filled out a baseline questionnaire, up front, and will complete subsequent questionnaires over the course of the study. Follow-up evaluations with each cooperating individual will be made every five years. Use information for pesticides and other risk factors is being collected in an attempt to relate exposure to disease. Cohort disease rates will be determined at regular intervals, and the probable causes of disease will be evaluated from information collected on the questionnaires. Finally, information on individuals diagnosed with specific diseases will be compared to information on those who are disease-free.

Advantages

A cohort study is the design of choice when studying what diseases may result from a specific exposure. The follow-up aspect of the cohort design provides very useful information on the interval of time between the first known exposure and disease detection: how long it takes for the disease to develop. Cohort studies are advantageous when the investigator wishes to evaluate a large number of diseases.

Limitations

Cohort studies are lengthy and expensive to conduct. A long-term commitment of resources and professional staff is required for the collection of accurate, useful information. Some diseases are so rare that a cohort of 100,000 people might be needed to yield adequate diagnosed cases to implicate exposure/disease correlation. Follow-up to end of study is difficult because individuals may move away and/or cease participation at any point.

Case-Control Design

The basic question addressed by case-control design is this: *Are individuals with diagnosed disease more (or less) likely to have been exposed than those without disease?* The distinguishing characteristic of case-control studies is that subject selection is based on disease status. Cases are identified among disease registries, hospitals' and physicians' records, and volunteers; disease-free members of the population that gives rise to the cases are selected as controls.

Exposure information is developed from existing records and/or from detailed questionnaires completed by the subjects. It is used to compare the frequency of exposure among cases and controls and to adjust, statistically, for other factors that may influence disease.

An example of a case-control study is one that investigates the likelihood that children with brain cancers were exposed to pesticides used inside or outside the home by their parents. Cancer registries are used for subject selection. Parents of both cases and controls are interviewed on their use of pesticides in and around the home, and the frequency of exposure (based on parents' recall) is compared.

Advantages

Case-control designs are extremely useful in studying uncommon diseases and those that take many years to develop. Since they start with individuals who have the disease, fewer subjects (than in cohort studies) are involved; and since the studies can be completed in a relatively short period of time (months or years) expense is often much less than for the cohort study.

Limitations

One major disadvantage to the case-control design is that information on exposure is collected *after* disease diagnosis. Diseased individuals may remember exposures or events differently than those who remain healthy. They also may be more highly motivated to participate in a case-control study.

Additional Study Designs

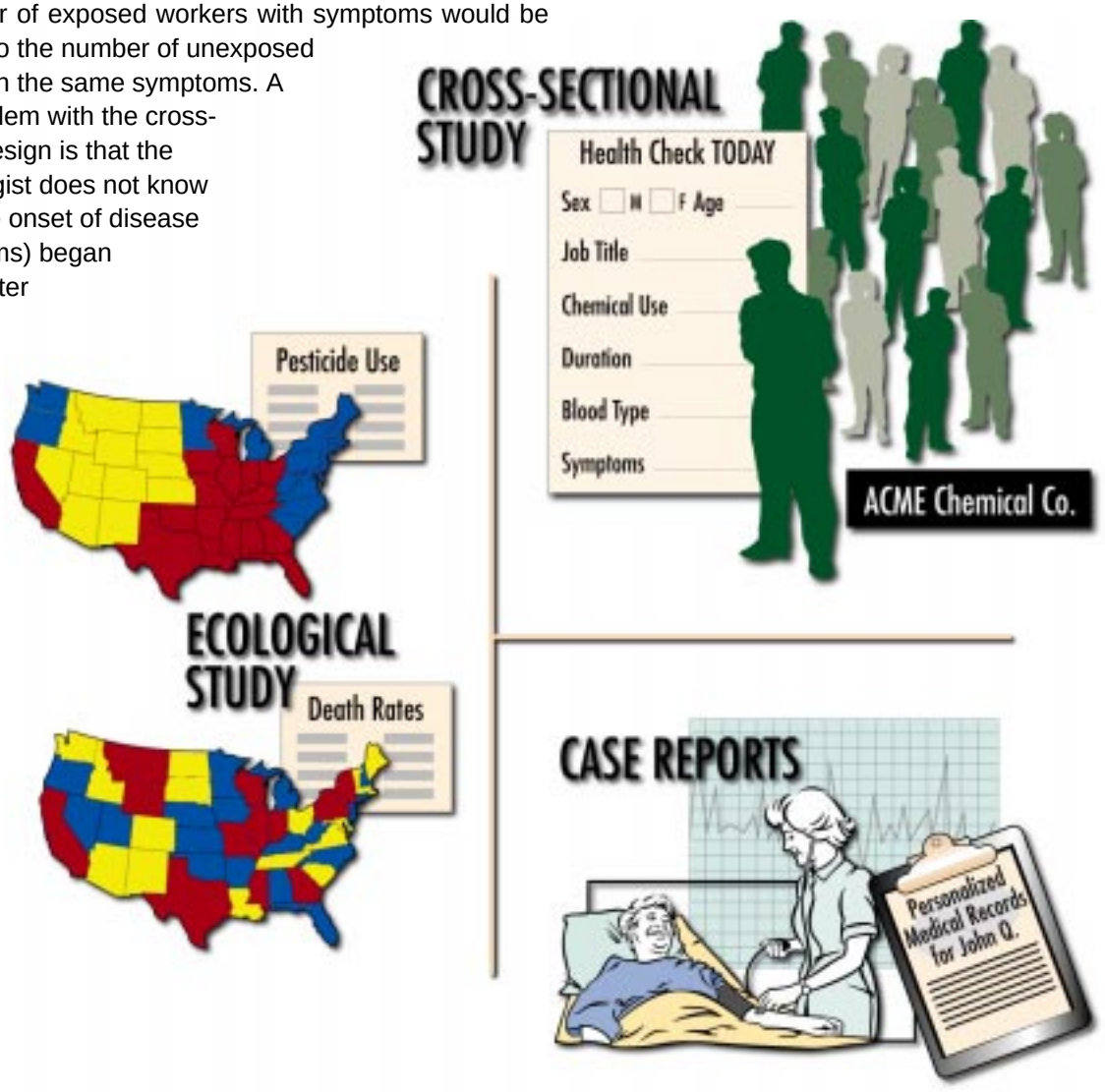
Epidemiologists can generate disease and exposure information from study types other than the cohort and case-control designs. These study types—case reports, cross-sectional studies, and ecological designs—are best used to develop hypotheses for more rigorous testing via cohort and case-control studies.

Case Reports

A case report is simply a description of a patient's diagnosis and disease progression, often published in medical literature by physicians who recognize a pattern or something unusual. Initial research on the correlation of pesticides to cancer stemmed from Swedish physicians' observance of a potential association between lymphoma (lymphoid tissue malignancy) and exposure to herbicides in patients diagnosed with the disease. Case reports provide no information on cause-and-effect, nor can they be extrapolated to larger populations. However, they are extremely useful in bringing forth observations, which alerts epidemiologists to the suspected relationship; this directs the focus of future studies toward specific disease/exposure relationships.

Cross-Sectional Study

The cross-sectional study simultaneously examines exposure and disease; that is, the epidemiologist starts with a defined population and, for each member of the population, collects exposure and disease information at (or from) a certain point in time. An example would be an investigation of pet handlers and health complaints. The handlers would be asked about their activities and products used. At the same time, they would be asked about a range of symptoms such as skin rashes and fatigue. The number of exposed workers with symptoms would be compared to the number of unexposed workers with the same symptoms. A critical problem with the cross-sectional design is that the epidemiologist does not know whether the onset of disease (or symptoms) began before or after exposure.



Ecological Design

Unlike case reports where individuals are described, and unlike case-control and cohort studies in which data are collected on individuals, ecological studies examine exposure and disease patterns for groups or populations. Generally, they utilize data that have been collected for other purposes. Hypothetically, an ecological

study might link data on mortality rates of non-Hodgkin's lymphoma (NHL) patients in certain Minnesota counties, as reported by the Minnesota Department of Health, to rates of herbicide use in the same counties as reported by the Minnesota Department of Agriculture. If high NHL death rates are recorded in counties where large amounts of herbicides have been applied, it is possible that exposure to herbicides is a causal factor. The problem with this design is that diseased individuals may not have been exposed to herbicides, a premise that cannot be substantiated because the data are available only for the population, not for individuals within the population. Epidemiologists are reluctant to base conclusions on ecological data; but, nonetheless, such studies can foster research hypotheses for future case-control or cohort studies.

Reporting Epidemiological Data

The presentation of epidemiological research in scientific journals and research reports varies with study design and the types of information collected. Following are some common methods for summarizing and presenting data in scientific literature.

Disease Rates

A disease rate is a measurement of the frequency of a disease, within a defined population, over a defined period of time. The frequency of disease is meaningless unless it can be defined with respect to the population involved and the time period of occurrence.

For instance, a one-year study reports that 93 children with birth defects were among 3,379 live births in a pesticide applicator population; in that same year, birth defects were detected in 1,493 children out of 68,493 live births among the general population. Superficially, it might appear that the general population experiences more birth abnormalities (1,493) than the pesticide applicator population (93), but raw number comparison distorts the proportion.

Epidemiologists address this problem by converting the number of cases found in a sample population to a common population size. For instance, the study recorded 93 birth defects among 3,379 live births within the applicator population and 1,493 birth defects among 68,493 live births within the general population. So it was necessary to convert the incidence of birth defects in both

the sample (applicator) and control populations to reflect a common population size, e.g., 1000. The raw numbers (93 and 1,493) are used merely as factors in the equation.

$$\frac{\text{93 birth defects}}{\text{3379 live births}} \times 1000 = \text{27.5 birth defects per 1000 live births for the applicator group}$$

$$\frac{\text{1493 birth defects}}{\text{68,493 live births}} \times 1000 = \text{21.8 birth defects per 1000 live births among the general population}$$

The focus of the research should be to compare the 27.5 birth defects per thousand live births among pesticide applicators with the 21.8 defects per thousand live births among the general population.

Rate Ratios

Rate ratios are comparisons of two rates, commonly used to measure disease associations between two populations. The two most commonly used rate ratios are the *relative risk ratio* (RR) and the *odds ratio* (OR).

Relative Risk Ratio

A measure of association calculated for a cohort study is a ratio called the relative risk (RR). It is a comparison of disease rates among exposed versus unexposed persons. It is often written as shown below:

$$\frac{\dfrac{\text{number with disease in the exposed group}}{\text{total number in the exposed group X years followed}}}{\dfrac{\text{number with disease in the unexposed group}}{\text{total number in the unexposed group X years followed}}}$$

An RR of 1.0 means that rates for a specific disease are the same for exposed and unexposed subjects. An RR exceeding 1.0 indicates a higher disease rate for exposed versus unexposed subjects, thus implying a possible relationship between exposure and disease. An RR less than 1.0 indicates a reduced disease rate in exposed subjects, possibly indicating a protective (or beneficial) effect of that exposure.

Example: An epidemiologist presents the results of a 20-year cohort study in which 3500 company employees—applicators, business managers, and office staff—had been enrolled. The incidence of lung cancer was of particular interest. The study identified 3000 employees from the cohort who, in the course of their employment, were exposed to insecticides; the remaining 500 were assigned jobs that did not bring them into contact with insecticides. Of the 3000 exposed employees, 32 were diagnosed with lung cancer. Among the 500 employees who were not exposed to insecticides, 20 were diagnosed with lung cancer during the same 20-year period. An RR of 0.27 was calculated for the prospective study, which means that individuals in the group exposed to the insecticide were less likely to develop lung cancer than those in the group who were not exposed.

$$RR = \frac{32}{3000 \times 20 \text{ years}} \div \frac{20}{500 \times 20 \text{ years}} = 0.27$$

Odds Ratio

The measure of association in a case-control study is the odds ratio. It is the ratio of the odds of exposure in the diseased group (case) to the odds of exposure in the nondiseased group (control). The odds ratio is analogous to the relative risk under most circumstances. Below is the formula for calculating the OR.

$$\text{Odds ratio} = \frac{\text{number of cases with exposure}}{\text{number of cases without exposure}} \div \frac{\text{number of controls with exposure}}{\text{number of controls without exposure}}$$

Example: An epidemiologist conducts a case-control study of prostate cancer, identifying (from the state cancer registry) 500 men aged 65 or older who had been diagnosed during the preceding two years; 1500 cancer-free controls were selected from a local population registry. Willingness to participate in the study was confirmed with individuals in both groups. Interviews and questionnaires were used to collect pertinent background histories (e.g., socioeconomic information) and to document past exposures based on the recollection of each participant. A total of 420 cases and 315 controls provided pertinent information. Analyses of the data indicated that 45 cancer cases had been exposed, at some time, to herbicides; and 20 of the controls likewise had been exposed, at some time, to herbicides. The epidemiologist calculated an odds ratio of 1.8 (see insert).

What does an OR of 1.8 mean? As with the RR, the baseline for comparison is 1.0. An OR of 1.0, by analogy to the cohort study, implies that the rate of disease is equal in exposed and unexposed subjects. In this example, an OR of 1.8 is interpreted to mean that the prostate cancer rate was 80 percent higher for exposed subjects than for those unexposed. When the OR is greater than 1.0, it suggests an elevated disease rate among exposed subjects. Conversely, odds ratios less than 1.0 imply a reduced disease rate for exposed subjects: The exposure may be protective.

$$\frac{45}{375} \div \frac{20}{295} = 1.8$$

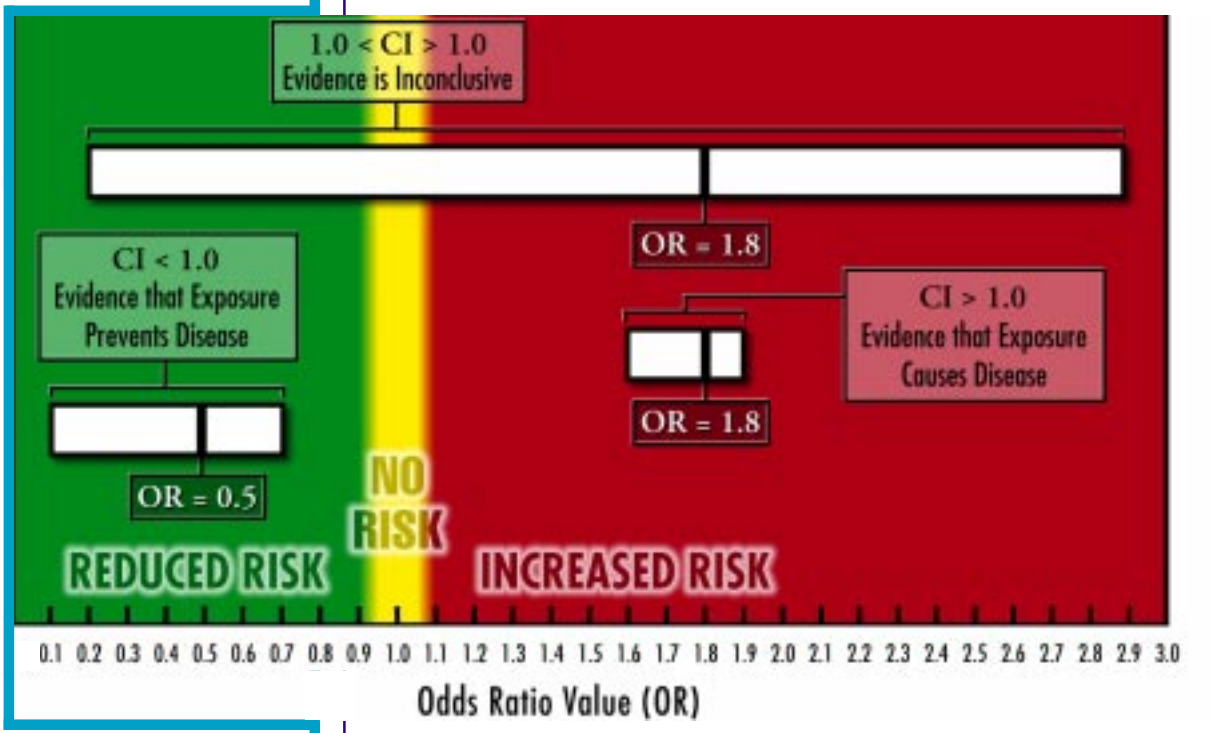
Confidence Intervals

The confidence interval is a valuable statistic that communicates information on the preciseness of the odds ratio and the relative risk. The odds ratio and relative risk are single point estimates of the ratio of disease rates for exposed and unexposed populations, but they are vulnerable to statistical variability; that is, the true value could be higher or lower than the point estimate. The confidence interval is usually constructed to provide the theoretical 95% upper and lower probability limits for the calculated OR or RR.

Epidemiologists typically use a 95% confidence interval (CI). For example, a report indicates that the OR is 0.9 and the 95% CI is 0.4 to 2.0. In this example, there is a 95% probability that the upper (2.0, an adverse effect) and lower (0.4, a protective effect) limits embrace the true estimate of risk in this population.

The confidence interval is very useful in judging the variability in the RR or OR. For instance, an epidemiologist reports an OR of 1.8 and a 95% confidence interval of 0.2 (lower) to 2.9 (higher) for the association between birth defect rates among the general population and commercial pesticide applicators. This is interpreted to mean that the rate of birth defects was elevated nearly twofold for

exposed subjects. However, the 95% confidence interval also indicates that the estimate could lie between the lower interval (0.2) and the upper limit (2.9).



If all of the values of the 95% confidence interval are greater than 1.0 (the level of risk) it could be concluded that exposure may cause the disease. However, if the 95% confidence interval includes 1.0 (e.g., 0.2–2.9) the results probably are inconclusive. As such, even though the OR or RR may exceed 1.0, the confidence interval means that the data do not clearly support the conclusion of an exposure-disease association.

Bias Complicates Study Results

The undoing of epidemiological research is bias, which is present to some extent in all human studies. Mistakes in planning, conduct, or analysis produce bias. Misclassification of individuals as exposed (or diseased) can result when subjects recall and report events differently. Bias also can occur when factors other than those being measured contribute to the disease or exposure. Bias can introduce error into information from which study conclusions are drawn.

Possible sources of bias are noted in well-conducted epidemiological studies. Potential pitfalls must be addressed before the results of a study can be considered valid evidence of a causal relationship. Following are types of bias that need to be minimized in all epidemiological studies.

Selection Bias

Selection bias occurs when there are major differences between the characteristics of people selected for the study and the characteristics of those who are eligible but not selected. For instance:

- Since control subjects do not have the disease under study, they may be less motivated (than the cases) to participate. For instance, if only 50 out of 100 individuals who are eligible to participate as controls agree to do so, the 50 participants may not reflect the underlying population that gave rise to the selected cases.
- Individuals with disease may be selected from a clinic where a high percentage of all patients treated have a significant characteristic or circumstance in common: migrant farm workers, for example. Controls selected randomly, by phone, would represent a much broader socioeconomic range, thereby invalidating comparison for purposes of the study.

- Occupational studies exclude persons who are too sick to seek employment; therefore, mortality rates for workers are lower than rates for the general population: the “healthy worker” effect.
- A sample of pest control operators would not be representative of all operators if the sample contained only workers who were allowed to take time off to participate in the study.

Information Bias

Information bias refers to mistakes in obtaining the necessary information on study subjects, such as a person being classified incorrectly with respect to exposure or disease. Perception of symptoms is highly variable among individuals; this is a problem when disease is self-reported, especially with subjective complaints such as headache, fatigue, and arthritis.

Self-reported exposure presents misclassification problems, as well. Using herbicides as an example, certain subjects will claim exposure if herbicides were applied to their lawn by a lawn service company, or if they walked on the sidewalk in a park where herbicide application signs were posted in the grass. Others would not consider either of the former situations actual exposures; in fact, some individuals report exposure only if they, personally, used the product. The truth may be that all, some, or none of the subjects were actually exposed!

Recall Bias

Recall bias occurs when subjects remember past events differently; it is selective recall influenced by disease status. Recall bias is of particular concern because epidemiologists must depend on the accuracy of information provided by respondents. An example of such a problem is in the study of birth defects. It is common for mothers who deliver children with abnormalities (case mothers) to wonder about the cause of birth defects by reliving every aspect of their pregnancies. When interviewed, the case mothers often recall the type and amount of pesticides and the number of times they were used in their homes or gardens or on their pets. By contrast, mothers with healthy births (control mothers) generally do not remember such details because they have no incentive to dwell on identifying probable cause of an unfortunate outcome such as a birth defect. Mothers of unhealthy babies may report higher frequencies of all kinds of exposure, suggesting, in this hypothetical case, a false exposure-disease relationship.

Recall bias is likely to be greater if the case is not available to be interviewed, that is, when the epidemiologist must interview a proxy (e.g., a daughter answering questions on behalf of her deceased father) to access case information. An example of this is

the reported use of the herbicide 2,4-D in a study of Nebraska farmers. Case subjects who responded for themselves did not indicate evidence of a relationship between 2,4-D use and the risk of non-Hodgkins lymphoma, whereas analyses based on proxy responses showed evidence of an exposure-disease association. The quality of detailed information from proxies is variable, although their responses to more general questions have been found more reliable.

Confounding Bias

Confounding bias occurs when the association between exposure and disease is distorted due to related extraneous factors. Establishing true relationships between exposure and disease often requires the epidemiologist to consider personal factors such as the age, gender, ethnicity, education, marital status, occupation, social class, and geographic location of each individual in the study. Age is the most important of the personal factors that influence disease. Incidence of chronic disease (e.g, cancer) generally increases with age. Accordingly, the effect of age must be accounted for when evaluating disease risks attributable to specific exposures; that is, the epidemiologist must remove the effect of the confounding personal factor in order to establish the true relationship between exposure and disease. Failure to account for personal factors can produce false associations ("finding" an association that does not exist) or obscure true causal associations (missing a relationship that actually exists).

Placing Epidemiological Studies in Perspective

The Individual Study

Epidemiologists communicate to the scientific community by exchanging project reports and by publishing their research in scientific journals. They frequently report on ongoing or recently completed research projects at professional conferences, government meetings, industry workshops, and public forums. While all of these forms of communication are important, it is the publication of epidemiological research in scientific journals that is the most important, primarily because journal articles are peer-reviewed

prior to publication. They are scrutinized by scientific experts on the subject matter, whose endorsement is required for acceptance into the journal. Scientists around the world accept published papers as contributions to their own research. Although publication in a scientific journal does not *guarantee* that the research results are accurate, it usually does indicate that peer researchers judged the research methods to be sound.

Understanding the research paper—the review process and the publication sections—and knowing how to judge it critically are of utmost importance in researching exposure/disease relationships.

The Independent Peer Review Process

The author submits a written manuscript to a journal and requests that the editor consider it for publication. The editor, in turn, submits the publication to one or more scientists familiar with the subject matter. The reviewers examine the study: objectives, designs, data acquisition, findings, and conclusions. These are blind reviews in that the author of the submitted publication does not know who is reviewing the manuscript. Anonymity allows the reviewer to honestly critique a study and freely express its merits or weaknesses. This process of independent and anonymous review is known as *independent peer review.*

The reviewers submit their recommendations to the editor, in writing. They can suggest that the manuscript be accepted for publication, as written; accepted with minor or major revisions; or rejected. The editor sends to the author all written comments, remarks, and suggestions from each anonymous reviewer. A manuscript that is accepted with revisions must be resubmitted with suitable resolution of the changes requested by reviewers. Authors do have the prerogative to present arguments as to why certain changes should not be made, and if the editor agrees with the author, he/she may overrule the reviewers.

This process, from the time the manuscript is submitted until it appears in press, often takes a year or more. Once published, the research paper is open to more scrutiny by a wider range of peers. It is then that the scientific community at large may review, criticize, and/or attempt to replicate the findings.

The Layout of the Scientific Paper

Author submission and publication of research findings follow specific guidelines established by journal editorial boards. While there are obvious differences in style and format among journals, the basic information is quite similar. The following sections are found in most journal publications.

Title

Titles of scientific papers are like titles of books: They need to convey the subject of the research.

Authors

The individual who heads a study usually is listed first on the publication, as senior author. The authors' affiliations (e.g., university, foundation, government) and addresses are referenced as footnotes in the paper, generally indicating which author to contact for reprints or correspondence. Funding sources for the research usually are presented as footnotes, as well.

Abstract

The abstract is the summary of the purpose, methods, results, and conclusions of a study. The information in an abstract should never be used alone, without reading the article, because the abstract omits important details and qualifications that may be critical to proper interpretation of the study.

Introduction

The introduction provides a brief synopsis of the more pertinent literature on the subject. The authors often cite scientific papers published in other journals, but on occasion they will make reference to other types of written materials: research theses and dissertations, manuscripts and reports, and personal communications. This information is used in the introduction to construct what is known and what questions have not been asked: gaps in the research. The questions that have not been asked often form the rationale for the research undertaken.

Materials and Methods

The materials and methods section is the meat and potatoes of any research paper: It tells how the subjects were selected, how the study was conducted, what measurements were taken, and how the data were analyzed.

Results

The findings of the study are presented in the results section. The written text is normally augmented by data presented in tables, figures, graphs, and charts. Important information found in this section include population characteristics, measures of disease

association (OR and RR), and their statistical precision. The results section should contain actual findings only.

Discussion

The discussion section in most journals is used by the epidemiologist to interpret study results. Authors frequently use their data and those from published literature to offer their professional judgment relative to exposure and disease association. This is a very useful exercise because it provides a forum from which the scientific community advances new theories and new ideas. It offers suggestions for further research.

References Cited

The references section provides the list of publications referenced in the text: author, title of the paper, journal and page number, and date. This is a very important part of any paper that merges past research with current; it directs readers to cited sources, facilitating their personal review of the references used by the authors to form their conclusions.

Documenting a Study and Its Findings

The validity of study results requires an accurate diagnosis of the disease; populations of exposed and unexposed subjects large enough so that meaningful differences can be isolated; assessment of the putative cause (etiologic agent); evidence of exposure (actual or estimated); and consideration of confounding factors and other biases.

It is important to understand that most epidemiological studies do not make causal inferences such as “this causes that.” Instead, they find statistical associations that suggest causal relationships. A statistical association does not necessarily imply causation; rather, it means only that the study has found that one or more factors appear related to disease.

Conversely, a nonstatistical association does not mean absolutely that a risk factor is not contributing to disease. Other factors not measured or accounted for may be masking or confounding researchers’ ability to recognize statistical association.

A major question to ask about epidemiological studies is whether an appropriate study design was used for the questions, hypotheses, and conclusions drawn by the epidemiologist.

Consider the following points when reviewing an epidemiological study:

- What kind of study design was used? Look for the research plan and, if present, the rationale for choosing one method of study over another.

• Were the objectives stated clearly, and was the study true to its objectives? Clear objectives should be presented for conducting the study. Research discussions should focus on what the study attempted to measure.

• Were the underlying assumptions and limitations of study design presented?

• How were the subjects selected? The logic for selecting or rejecting individuals in any study should be articulated, describing the specified populations from which subjects were drawn and the methods used in their selection. It is the most important point that a study must address if the results are to have significant meaning.

• Were exposures and medical outcomes assessed using objective and reasonably accurate procedures?

• Did the study include an appropriate control or comparison group?

• Was the rationale and criteria for inclusion and exclusion of cases and controls presented?

• Was the rationale and criteria for disease ascertainment and exposure classification discussed?

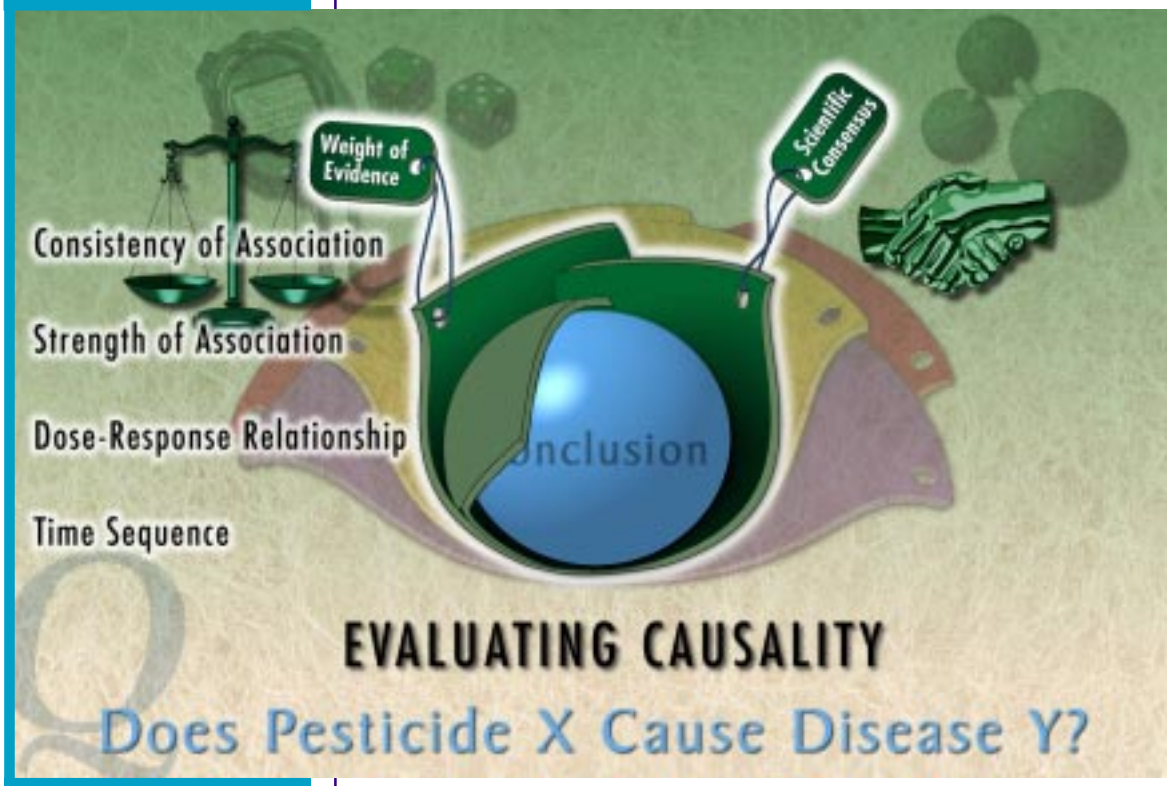
• Did the study find differences between groups for the stated hypotheses? More confidence is placed in a study that finds differences in the pre-specified set of questions or hypotheses proposed in the introduction. Less confidence is placed in unexpected findings. More confidence is placed in findings that make clear biological sense and have been replicated.

• May confounding variables explain the association? Scientists often ask whether the rise in one factor (exposure) which gives rise to an outcome (disease) is actually dependent on another variable that was not measured.

• Were some of the results inconsistent with the conclusions of the authors?

Weight-of-Evidence From All Sources

A single study, except under extraordinary circumstances, cannot establish a cause-and-effect relationship. It is necessary to link all of the studies as pieces of a puzzle to see how they fit together. The ultimate causation judgment of the scientific community should be based on a weight-of-evidence approach.



Hill's Criteria

A widely used method for reviewing scientific evidence involves applying Sir Bradford Hill's criteria for causality. Hill's criteria emphasize the necessary precedence of exposure to disease, the size of the risk estimate (RR or OR), and whether the disease rate or risks increase or decrease with increased exposure. Hill's criteria include discussions on the following points.

Biological Plausibility

Biological plausibility is inherently judgmental and limited by our current knowledge of basic disease processes. Can a biological mechanism be shown to explain how a particular agent could have caused the disease? Are there biological explanations that link exposure pathways and disease pathology? Do clinical or laboratory observations fit the findings from population study?

Time Sequence

The appropriate time relationship between first exposure and disease detection must be demonstrated. Each disease requires a certain length of time between environmental exposure and the manifestation of disease in humans. For example, associating a

risk factor with cancer usually requires years or decades consistent with the length of time that most cancers require to become clinically evident.

Dose-Response Relationship

A dose-response relationship is also an important component in Hill's criteria. The RR should increase or decrease as the level of exposure increases in order to satisfy this criterion. Persons exposed at high levels should experience greater effects than those exposed at lower levels.

Strength of the Association

The strength of association for each risk factor is a major consideration, in that it relates to confidence intervals and sample size. For example, a relative risk of 4.0 is given more weight as a potential causal factor than a relative risk of 2.0 in a similar study. An odds ratio of 1.2 is less convincing than an odds ratio of 5.0—and even this is dependent on the variability.

Consistency of the Association

An association between exposure and disease generally needs to be demonstrated in several similar studies before epidemiologists begin to consider causality. Research errors and chance findings do happen, and history has shown the scientific community that it is easy to be misled by an apparently sound (but isolated) finding.

Statistical Association

Statistical significance testing is a tool used to objectively evaluate the role of chance or sampling variation in the observed findings of the study. Conventionally, epidemiologists have considered results with a probability value (P value) of less than 0.05 to be statistically significant. In other words, under ideal circumstances, findings as extreme or more extreme than those observed in a study have less than 5 chances in 100 of occurring due to chance. Over the years, many scientists have used statistical significance as a decision rule for separating valid from invalid findings, but this practice has fallen into disfavor for two reasons. First, when there is bias in a study, significance probability calculations are misleading. Second, an exposure/disease relationship may be truly causal but not statistically significant, due merely to a small study population. Accordingly, the use of statistical significance should be viewed only in the context of the other strengths and weaknesses of the study.

Scientific Consensus

Consensus is often sought by governmental agencies, medical communities, and other public organizations that are considering reducing exposures or allocating funds for public education. Panels are assembled to discuss the available findings and derive a consensus statement or conclusion. Such organizations frequently use Hill's criteria as a point of reference, but they may place more emphasis on the subjective opinions of committee members.

It is important to know that these opinions may or may not be reflective of the views of the broader scientific community. In many instances, the panel's findings may subsequently change scientific consensus. There are many examples where not all of the members of a consensus committee agree on major issues. This often leads to publishing not only a majority report, but a minority report, as well. Those in support of the minority report make their case as to why they deem the majority report erroneous.

CONCLUSION

Epidemiologists and the medical community are increasingly researching the potential human health effects of pesticides outside the laboratory setting. This is important because epidemiological studies can provide information that cannot be predicted from testing on nonhuman species. Studying the effects of human behavior, as well as multiple exposures under real world conditions, adds to the toxicological evidence derived from laboratory studies.

The new information derived from these studies becomes available to the public in bits and pieces. It requires great care on the part of the media and others not to exaggerate preliminary findings from single studies. Epidemiological evidence needs to be viewed with a critical eye because of limitations inherent in any study. This holds true regardless of the completeness, accuracy, or objectivity of the press or the investigators in the study.

Even experienced senior epidemiologists have difficulty interpreting some epidemiological findings. Those not trained in epidemiology (e.g., the media) face additional difficulty due to their incomplete understanding of the field; and that difficulty is compounded by the fact that news reports of epidemiological studies are *abbreviated* versions of scientific journal articles. Often, the news media misconstrue or overemphasize certain findings without mention of the authors' own scientific disclaimers. This is why

importance is placed on obtaining the original published work to review for yourself the evidence presented in the publication.

The nature of epidemiology as a science lends itself to difficulty in studying health effects of pesticides. Indeed, each human is unique, their behavior unpredictable. Studying groups of people to determine if exposure to pesticides causes disease is a challenging task. Nonetheless, the science of epidemiology has contributed

significantly to the understanding of human health risks from exposure to pesticides. Studies that have shown a relationship have led to some products being replaced by safer ones. Other data have been used to set exposure guidelines for manufacturers or professional applicators. Studies showing no adverse health relationships have increased our understanding of the potential benefit of pesticides and directed research toward other possible causes of disease. Collectively, the goal is to reduce risk associated with human exposure to pesticides and to maximize benefits from their use. Epidemiology plays a major part in this goal.

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