Epidemiological Studies of Neurotoxic, Reproductive, and Carcinogenic Effects of Complex Mixtures

Carl M. Shy

Department of Epidemiology, School of Public Health CB7400, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7400

Neurotoxic, reproductive, and carcinogenic effects are potentially important health endpoints in epidemiological studies of complex mixtures, particularly when such mixtures contain volatile organic compounds or trace metals. Epidemiological studies of neurotoxicity often will require direct clinical, behavioral, and/or physiological testing of study subjects, because these effects are likely to be subtle and not identifiable as clearly defined diseases. Peripheral nervous system toxicity can be assessed by clinical neurologic examinations, by electrophysiological tests of nerve conduction, and by physiological tests of thresholds for neurosensory perception, though these tests require considerable standardization for use outside the clinical setting, and most of the available tests have not been assessed for their utility in detecting effects of neurotoxic exposures. Neurobehavioral effects of exposures to solvents, as examples of complex mixtures, have been studied widely; but batteries of tests are often used, and these have not been well standardized and are generally unfamiliar to most research investigators in this area. Recently standardized neurobehavioral test systems developed by the World Health Organization and by a U.S. group for use in field studies, show promise in detecting neurobehavioral effects at relatively low environmental exposures. Similarly, new and sensitive measures of disturbed reproductive function, such as time-to-conception and biochemical indices of early pregnancy loss, are affected by some low-concentration environmental agents; but those measures have not yet been applied to studies of complex mixtures. Because of the long latency problem and small expected relative risks, population-or community-based studies of the carcinogenic effects of complex mixtures are unlikely to yield data of adequate quality to justify more than exploratory studies of carcinogens in ambient air. — Environ Health Perspect 101(Suppl 4):183-186 (1993).

Key Words: Neurotoxicity, reproductive effects, cancer, indoor air, air pollution

Introduction

The objective here is to consider the feasibility of performing and using epidemiological studies of neurotoxic, reproductive, and carcinogenic effects in populations to assess the human health consequences of exposure to indoor air and other complex mixtures of air pollutants. The rationale for considering effects other than nonmalignant respiratory effects of air pollutants may be quite obvious but worth expressing; The primary effect of the most commonly studied air pollutants, such as fine particulates and ozone, is directly on respiratory tissue. However, carbon monoxide and lead are two primary air quality pollutants that affect other organ systems. Mixtures of air pollutants containing these compounds or other trace metals or volatile organic compounds also are likely to induce nonmalignant effects in organ systems other than the respiratory. Epidemiological tools are now reasonably available to study neurotoxic, reproductive, and carcinogenic effects. Effects on other tissues or organ systems such as the liver, kidney, skin, endocrine, and immune systems also are possible, but considerable methods development is necessary before these categories of effects can be studied systematically in populations.

Neurotoxic Effects

Sources of Data

Unlike cancer and birth defects, there are no regional or national registries for neurological diseases. Medical records from hospital admissions or medical insurance claims may be acceptable sources for studies of environmental factors in the etiology of defined diseases such as Parkinson’s or Alzheimer’s, but the major problem in epidemiological studies of these chronic neurological disorders is the probable latency between etiological exposures and disease manifestation. Some neurotoxic effects, such as disturbance in cognition or in nerve conduction, may be linked closely in time to environmental exposures, but these effects are likely to be subtle and insidiously manifested, and they are not identifiable as clearly defined diseases. Acute pesticide poisonings, usually due to organophosphate toxicity, are reported in some states, but reported episodes are thought to underestimate greatly the true incidence of such events. In general, in contrast to cancer and some reproductive effects, epidemiological studies of neurotoxicity induced by complex mixtures will require direct clinical, behavioral, and/or physiological testing of study subjects by the investigator. Exceptions to this general statement are potential case-control studies of defined clinical neurological diseases such as Alzheimer’s, Parkinson’s, and other entities, though there is little likelihood that retrospective estimates of determined exposures to complex mixtures could be satisfactorily for studies of such diseases.

Range of Neurotoxic Effects and their Measurement

For this discussion, it is assumed that typical ambient and indoor concentrations of complex mixtures containing neurotoxic components will not cause acute toxicity, but rather that the investigator will attempt to evaluate the effect of low concentrations of agents such as solvents, agricultural chemicals, other volatile organics, or trace metals in the indoor or ambient environment. Two primary classes of neurotoxic effects can be studied in population groups: peripheral nervous system toxicity, and neurobehavioral impairment reflecting central nervous system toxicity. Peripheral nervous system toxicity can be assessed by clinical neurological examination, by electrophysiological tests of nerve conduction velocity, and by tests of...
thresholds for neurosensory perception (e.g., visual, hearing, odor, and cutaneous vibration thresholds). The clinical neurological examination has the disadvantage of requiring highly skilled clinicians who are notoriously difficult to standardize in their procedures, and the results are only semi-quantitative at best. There is no evidence that clinical examinations have detected early effects of neurotoxic exposures. Tests of nerve conduction velocities are fully quantitative and can be performed by trained technicians using standardized techniques. Such tests have been used to detect early effects of increased lead exposures among workers (1,2). A distinct disadvantage of nerve conduction tests is that they can be uncomfortable and therefore poorly received by test subjects. Tests for changes in hearing thresholds are very common and well standardized but have not been used often in epidemiological investigations of potential neurotoxic exposures. Tests of thresholds for visual, odor, and cutaneous vibration thresholds (3) are used less frequently, but the potential for standardization and use by trained technicians is clearly present. There is as yet little data for assessing the utility of neurosensory threshold tests for detecting the effects of environmental neurotoxin exposures.

The neurobehavioral effects of exposure to solvents (4-6) and to lead (7,8) have been evaluated in a considerable number of epidemiological studies. A wide range of impairments can be addressed, such as alterations in memory, learning, cognition, mood, attention, and neuromuscular performance (e.g., eye-hand coordination). Neurobehavioral function can be studied by means of questionnaires and by objective, physiological, and psychological tests. A major problem with their use in epidemiological studies has been a lack of standardization of test batteries and a lack of familiarity with the use of these tests for field studies. These problems are being addressed, however, at the national and international level. The World Health Organization (WHO) (9) has developed a well-validated neurobehavioral test battery, the Neurobehavioral Core Test Battery, comprising measures of auditory memory, affect, manual dexterity, visual perception and memory, attention and response speed, and perceptual motor speed. The disadvantage of the test battery is that it requires 50 min per subject and a highly trained test administrator. A second test battery has been developed in the United States (the Neurobehavioral Evaluation System [NES]) by Baker et al. (10). This battery tests the same functional domains as the WHO Core Battery, requires the same length of time, but is computerized and thus does not require the presence or administration of a highly trained test administrator. The NES holds great appeal because multiple subjects can be tested simultaneously and results are scored and tabulated immediately by computer linkage.

**Epidemiological Applications**

Cross-sectional studies of lead-exposed workers have shown a variety of neuropsychological effects quantitatively related to blood lead levels (11-15). Needleman et al. (16) and Bellinger et al. (17) performed longitudinal studies on the growth in cognitive function of children exposed to relatively low to moderate ambient lead concentrations; these studies provide evidence for small but important decrements in the development of cognitive and neurobehavioral function in children who had normal to high-normal blood leads in the first years of life. Relatively few longitudinal studies of lead-exposed adults have been reported. One example is a study by Baker et al. (7) suggesting an improvement in neurobehavioral function in a cohort of workers exposed to lead at a foundry in which hygienic conditions were significantly improved.

Several reports from Scandinavia (18) illustrate epidemiological studies designed to assess the long-term neurobehavioral effects of complex mixtures, including carbon disulfide in textile plants and solvents in paints. In several studies, psychomotor and intellectual functions of exposed workers were affected, and on the whole, higher intellectual functions seemed to be more affected by exposure than psychomotor performance. Gamberale (18) notes that while some aspect of neurobehavioral performance was found to be impaired in the great majority of studies, differences in test results between exposed and nonexposed groups of workers sometimes disappeared when the groups were matched on intellectual level. Differences still persisted in tests of reaction time, which are not correlated with intellectual level.

Because our focus of concern here is on low-level indoor and ambient exposures, it is more likely that serial tests of the same persons will be necessary to detect neurotoxic effects. Investigators will have to identify population groups, particularly workers, in whom preexposure and postexposure test results can be compared. Alternatively, results obtained during and after termination of exposure may be compared. This strategy avoids some of the serious selection biases encountered in cross-sectional studies of populations, in which exposure can induce selective loss of susceptible persons. A distinct advantage of neurotoxic studies is that the tests may be sensitive enough to detect effects with a minimal latency, thus allowing estimates of exposure to be made from concurrent measurements. Like cross-sectional studies, case–control strategies have the inherent problem of addressing past exposures; and for many neurological impairments, cases are difficult to identify from medical records.

**Recommendation**

Because volatile organic compounds and trace metals may often appear as components of complex mixtures and because these substances include known or potential neurotoxins, neurotoxic effects should be evaluated intensively in exposed populations. Standardized test batteries are now available for such studies, but they have not been used widely; and although standardized, their efficacy for evaluating low-level neurotoxic exposures has not been validated. The topic is broad and important enough to warrant its own workshop. Multiple issues need to be addressed, such as training of neuroepidemiologists, dissemination of information on the availability and applications of neurotoxic testing, methodological studies to determine whether some subsets of the lengthy test battery are appropriate particularly for low-level combined mixtures, and requests for applications (RFAs) to foster interdisciplinary epidemiological studies of neurotoxic exposures. Neuroepidemiology is not a developed subspecialty of epidemiology compared with levels of activity in the developing areas of biochemical, reproductive, and pharmacoepidemiology, to say nothing of the well-developed fields of cardiovascular, cancer, and infectious disease epidemiology. Several federal agencies have a legitimate reason for promoting the development of neuroepidemiological research; these include the National Institute of Environmental Health Sciences (NIEHS), National Institute of Neurological Disorders and Stroke (NINDS), United States Environmental Protection Agency (EPA), National Institute of Occupational Safety and Health (NIOSH), and Agency for Toxic Substances and Disease Registry (ATSDR). The Health Effects Institute could bring together representatives from these agencies, along with appropriate academic disciplines, in a workshop on the development and use of neuroepidemiological studies of environmental exposures.
Reproductive Effects

Source of Data

Several states now have birth defect registries. Some reproductive outcomes, such as birth weight and gestational age, are listed on birth certificates. Hospital and clinic records are sources of information on clinical spontaneous abortions, complications of pregnancy, (preclampsia, abruptio placenta, etc.), length of gestation, APGAR scores at birth and overt congenital defects at birth. An increasing number of ultrasound tests and amniocenteses are being performed in utero, and these tests provide additional data on fetal development.

Range of Reproductive Effects for Use in Epidemiological Studies

Although birth defects, birth weight, and clinical spontaneous abortions are the most frequent reproductive outcomes considered in epidemiological studies, they may not be sensitive to low-level exposures. Detecting changes in birth defect rates requires very large study populations, and thus studies of environmental exposures and birth defects are often infeasible. Birth weight is a quantitative outcome and, as such, provides adequate sample sizes for detecting small effects. However, racial and socioeconomic factors are strong epidemiological determinants of birth weight, and there is little evidence that environmental chemicals contribute to variations in the population distribution of birth weights. Clinical spontaneous abortions probably reflect less than half of all spontaneously terminated pregnancies; epidemiological methods now exist to detect very early pregnancy losses but have not been applied to study environmental exposures. Several studies have used time-to-conception as a quantitative measure of subfecundity and have demonstrated an effect of cigarette smoking (19) and of the anesthetic gas, nitrous oxide (20). Disturbances of menstrual cycles and of sperm counts and sperm mobility are known to be induced by environmental factors, but these effects measure also are relatively new and are not validated. Overall, rapid developments are taking place in the reproductive biology disciplines, and these are providing new tools for epidemiological assessment of reproductive effects. Several of these tools are now ready for application to populations exposed to potential reproductive toxins in the environment, and the number of qualified investigators and of studies in progress indicates that this will be a fruitful area of research throughout this decade. The entire spectrum of reproductive effects from preconception events through conception, pregnancy, and early childhood development is essentially unexplored in terms of sensitivity to environmental exposures.

Recommendation

Epidemiological measures of reproductive effects are available, and their utility in epidemiological studies has been well demonstrated (21). On a national scale, more effort is being put into reproductive epidemiology than into neuroepidemiology. However, the newer and potentially more sensitive measures of reproductive function, such as biochemical indices of early pregnancy loss and menstrual cycle disturbances, have not been applied to environmental exposures. Some distinct advantages of these endpoints are that latency is short, contemporaneous exposures are relevant, and they are common events. Although laboratory research on specific exposures and reproductive outcomes is clearly desirable, human studies need to proceed simultaneously because environmental complex mixtures cannot be readily reproduced in the laboratory, and variations in human versus animal susceptibility are not understood well enough to make confident extrapolations from animal models. Therefore, applications of the newer and potentially more sensitive measures of reproductive outcomes to selected population exposures should be encouraged. Methodological and applied epidemiological research is needed across a broad range of exposures.

Cancer

Sources of Data on Cancer Incidence and Mortality

The majority of states now have statewide cancer registries, though many of these are in the first years of implementation. Tumor registries at teaching hospitals and medical records at all hospitals are important data sources for case-control studies of cancer risks. However, the major obstacle to effective use of these registries and data bases is the time lag between first diagnosis and entry of cases into the registry. To obtain useful information on environmental and occupational exposures of cases, often it is necessary to interview cases directly and to do so while they are still able to be interviewed. Thus, the time lag between diagnosis and interview is critical, and requires case identification more rapidly than most cancer registries function. Ideally, cases would be interviewed while they are still in the hospital for their first evaluation. Methods have been used at some research centers to administer standardized questionnaires to all patients admitted for a cancer work-up, and these data have been fruitful for exploring various environmental risk factors. However, the most abundant source of evidence on specific environmental risk factors has been industrial cohort studies in which exposure estimates are based on the work history of cases and controls as recorded in the historical personnel record of employees.

Source of Data on Exposure to Carcinogens in a Complex Air Environment

With a few exceptions, population or community-based studies in contrast to occupational cohort studies are unlikely to yield exposure data of adequate detail or quality to justify more than exploratory studies of carcinogens in ambient air. The major obstacles to definitive studies in the general community environment are long latencies; small expected relative risks; a low proportion of the population exposed to specific and clearly defined complex mixtures; difficulty in estimating these exposures for past decades with any degree of specificity; and high mobility of populations between various home, work, and geographical environments. A few reasonably common and measurable exposures to environmental carcinogens (proven or potential) have been assessed in community studies (e.g., indoor radon, electromagnetic fields, and environmental tobacco smoke). Most complex mixtures in indoor or ambient air are unlikely to be as common as these three risk factors or as amenable to drawing distinctions between exposed and nonexposed persons in the community environment. The most feasible epidemiological investigations of complex mixtures containing carcinogens are likely to be studies of defined occupational cohorts, among whom at least qualitatively distinct exposure groups can be identified and for whom records of cumulative exposure are available. A relatively recent example is the NIOSH study of laryngeal cancer and acid mist exposures of steel workers involved in pickling operations (22). While occupational cohort and case-control studies offer the greatest promise for analytical epidemiological studies of complex mixtures, there is a need to develop and apply to exposed community-populations studies of the occurrence of biological markers of carcinogenic exposure. Typically, communities adjacent to toxic waste sites or to point sources that process toxic wastes, such as incinerators and chemical treatment facilities, are concerned
about cancer risks. Epidemiological studies of cancer risks per se are practically infeasible in these environments due to inadequate populations at risk, undefined exposures, and short time since first exposure. However, it is feasible to study the biological uptake of some of the associated compounds in these populations. Thus a battery of biological markers might be a useful epidemiological tool to evaluate the potential for human exposures at these sites.

**Recommendations**

Occupational cohorts should be characterized with respect to exposures to carcinogenic complex mixtures that occur in indoor or ambient air environments. These cohorts should be the first choice for epidemiological studies of the potential carcinogenic effect of these mixtures.

Batteries of biological markers of carcinogenic exposures should be developed for use in epidemiological evaluations of populations exposed to point sources of complex mixtures containing suspected human carcinogens.

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