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A Reproductive Hazards Research Agenda for the 1990s

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There is substantial scientific and public concern about the potential effects of occupational and environmental toxicants on reproductive health. These effects include impaired functioning of the reproductive systems of men and women as well as a broad spectrum of developmental problems expressed in offspring. Research on reproduction and development is among the most complex undertakings in biomedical research. This complexity is due in part to the intricate biology of reproduction, the multiple targets involved (male, female, and offspring), the uncertainties in extrapolating from animal models to humans, and the problems involved in accurately characterizing exposures and outcomes in epidemiologic investigations. However, given the relatively brief history of research into toxicant-induced reproductive health effects, we have made enormous strides in our knowledge over the past decade. In particular, recent advances in reproductive biology and biotechnology and in the development of biological markers of exposure, effect, and susceptibility are greatly enhancing our ability to study cause–effect relationships. In this paper, the Research Needs Working Group proposes ways to apply existing knowledge to better protect reproductive health and suggests directions for future research. Fulfilling this challenging agenda will require responsible cooperation by labor, industry, government, individual citizens, and the scientific community. Further research and collaboration are essential to both prevent adverse reproductive and developmental outcomes and to formulate a sound scientific basis for policy making.

Introduction

Reproductive health policy decisions should protect our reproductive health, civil rights, and economic opportunities. Exposures to men and women in the workplace and the general environment are of concern. Policy decisions should be guided by a clear knowledge of the factors that can result in reproductive harm as well as those that pose little or no risk. Unfortunately, in the area of reproductive risk, the scientific knowledge needed to provide clear guidance to policymakers in the public or the private sector is frequently lacking.

The purpose of this paper is to summarize some of the difficulties involved in assessing reproductive and developmental risk potentially associated with exposure to toxicants in the workplace or environment. We also describe some promising new developments in the assessment of reproductive hazards. The paper concludes with policy recommendations and a research agenda agreed upon by participants in the Research Needs Working Group at the Woods Hole Conference.

Scope of the Problem

Environmental hazards to reproduction and development are a source of substantial scientific and public concern. In the 1960s, the use of thalidomide in Europe and environmental contamination by methyl mercury in Japan made the possibility of birth defects from drug and chemical exposure tragically apparent. Early in the 1970s, diethylstilbestrol was recognized as causing adenocarcinoma of the vagina in young women whose mothers had taken the drug during pregnancy (*1*). Later in that decade, it was discovered that men occupationally exposed to the nematocide dibromochloropropane (DBCP) were subject to varying degrees of testicular toxicity culminating, in some instances, in infertility (*2*). These and other examples of adverse effects on reproduction, coupled with an

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increase in the number of couples seeking treatment for infertility (due primarily to delayed childearing) and an increase in the number of women in the workforce during their childbearing years, have intensified public concern.

In considering reproductive and developmental toxicity, we include impairment in the functioning of the reproductive system of males and females (which may be evidenced by the inability to reproduce) as well as toxic effects expressed in the offspring. Environmental agents may act at several stages during reproduction and development which include complex, interdependent processes involving the nervous, endocrine, and immune systems.

The extent of reproductive dysfunction and adverse pregnancy outcomes in the United States is significant. According to currently available data, 1 in 12 couples of reproductive age is infertile, that is, they are unable to produce a viable pregnancy during 1 year of unprotected intercourse (*3*). Ten to twenty percent of recognized pregnancies end in pregnancy loss and at least as many conceptuses are lost prior to recognition of the pregnancy (*4*). Low birth weight, a major contributor to perinatal morbidity and mortality, affects approximately 7% of all newborns and more than 13% of black infants (*5*). Two to three percent of liveborns have major congenital malformations recognized at birth. More have malformations diagnosed as they grow older (*6*). It is not clear how many children suffer from developmental delay or other functional deficits. Other problems of the reproductive system that affect individuals of either sex include sexual dysfunction, hormonal imbalances, and alterations in reproductive maturity or senescence. Systematic data on the prevalence of these end points are lacking.

The extent to which environmental and occupational exposures to men and women contribute to the burden of reproductive and developmental dysfunction is unknown. Only a small proportion of the more than 60,000 chemicals in commerce today have been adequately tested for reproductive or developmental toxicity. Not only are chemical agents of concern, but biological and physical agents as well as psychological stress are capable of interfering with reproductive processes. Only a handful of agents are generally recognized human reproductive toxicants. This small number reflects, in part, the difficulties of identifying causal associations in humans as well as the lack of data.

Research Advances and Limitations

Research on reproduction and development is among the most complex undertakings in biomedical research. This complexity is due in part to the fact that more than one organism is involved in many aspects of reproduction (the male and female) and development (both parents and the offspring). In addition, most current knowledge about reproductive toxicity derives from studies of experimental animals whose anatomy, physiology, and metabolism of xenobiotic agents may differ from those of humans. Since animal studies must continue to play a critical role in reproductive toxicity testing, more information is needed on the predictive value of animal experiments for humans.

Recent advances in reproductive biology and biotechnology (exemplified by *in vitro* fertilization) have increased opportunities for the study of a wide range of reproductive end points, including subtle effects of exposure to environmental toxicants. Biological markers for assessing very early pregnancy loss, endocrine function, reproductive senescence, male reproductive capacity, and genetic susceptibility have been developed and are beginning to be used in epidemiologic studies of environmental exposures (*7*). These sensitive new tools promise to greatly enhance our ability to study cause–effect relationships in reproductive epidemiology.

Valid results in the epidemiologic study of reproductive and developmental risks depend on our ability to precisely ascertain exposure and outcome. Unlike the development of chronic diseases, where total duration of exposure is typically the most relevant exposure parameter, effects on reproduction and development are often the result of short-term exposures during critical periods of vulnerability. Fetal organogenesis, which occurs primarily during the first trimester of pregnancy, is one such vulnerable period. Ovulation and spermatogenesis are others.

In some cases, the result of a toxic exposure may not become evident until years later. This can occur by at least three distinct mechanisms. First, some toxicants bioaccumulate in parental tissues and may be released during pregnancy, lactation, or spermatogenesis. For example, polychlorinated biphenyls (PCBs) are stored in adipose tissue and lead is stored in bone. These compounds can be released from parental tissues many years after exposure has occurred. Second, toxicants that deplete the number of female germ cells may result in a shortened reproductive life span, which would not be evident until senescence. For example, it has been shown in rodents that polycyclic aromatic hydrocarbons (PAH), components of cigarette smoke, can be lethal to oocytes (*8*). This may explain the earlier menopause observed in women who smoke cigarettes (*9*). Finally, toxic effects to offspring may not become apparent until a particular stage in postnatal development. For example, neurobehavioral or carcinogenic end points in developing offspring can follow maternal or paternal preconceptional exposure.

One of the most difficult tasks in reproductive epidemiology is accurately assessing exposure. Frequently, occupational studies must rely on job titles to impute exposures. Misclassification of exposure by this method can be substantial (*10*). The same job title may be associated with vastly different job tasks or exposures in different companies or locations. The impact of using an inaccurate measure of exposure (if unbiased) is toward obscuring a true effect. Improvements in industrial hygiene assessments of occupational exposure, as well as the development of biological markers of exposure, show great promise for improving our ability to quantify exposure in biologically relevant tissues.

Ascertainment of outcome in the study of reproductive and developmental risks is also problematic. Definitions of spontaneous abortion, congenital malformations, semen abnormalities, and developmental delay are not consistently applied in clinical practice. National surveillance

data are not available for any of these outcomes. Patterns of access and utilization of health care services also affect the reporting of adverse reproductive or developmental outcomes. For example, many early spontaneous abortions remain undetected, since women do not seek clinical services for these outcomes. Many people with fertility problems may not seek medical intervention due to lack of adequate health care coverage.

In spite of these difficulties, it is in the area of outcome assessment that the greatest strides have been made in recent years. For example, it is now possible to study the DNA in fetal cells and in sperm, evaluate aspects of endocrine function with spot urine samples, detect pregnancy around the time of implantation (about a week after fertilization), and detect subtle neurodevelopmental dysfunction.

However, the clinical relevance of subtle changes (e.g., in neurobehavioral, semen, or endocrine parameters) still needs to be determined.

Individual biological responses to a toxicant may be modified by demographic, nutritional, or genetic factors, as well as by health status and concomitant exposures. Deficiencies of iron, zinc, and calcium, for example, are associated with increased gastrointestinal lead absorption (*11*). The principle of genetic variability in response to xenobiotic exposure is well established, and evidence is accumulating to suggest genetic influences on teratogenic outcomes. For example, recent evidence suggests that the risk for alcohol-related birth defects may be affected by genetic polymorphisms of alcohol dehydrogenase, the rate-limiting enzyme in alcohol metabolism (*12*). Beyond these data, very little is known about variations in susceptibility to reproductive or developmental toxicants or about synergistic effects of multiple exposures.

Scientific research into the reproductive and developmental effects of occupational and environmental chemicals started relatively recently. Given its short history, the advances that have been made in the field are impressive. However, we still have much to learn about reproductive and developmental hazards and need improved methods to identify potential toxicants. In the meantime and despite our best efforts, cases will continue to arise where the current science of reproductive hazards is simply inadequate to meet the more immediate needs of policymakers or concerned workers and citizens. Given the many years of study required to suggest cause–effect relationships, scientists will most frequently not have clear-cut answers to the questions asked by these sectors. Understanding the limitations of the currently-available scientific evidence, while continually trying to improve upon them, is an essential part of the job of scientists, policymakers, and concerned individuals if we are to derive maximum benefit from research on reproductive and developmental toxicants.

Recommendations

Policy Recommendations

The critical scientific evaluation of reproductive and developmental toxicity data should be undertaken on an ongoing basis by a suitable national or international agency. This agency should determine which agents require immediate action and prioritize agents requiring further research. Agents for which there are data regarding exposure of one sex only should be placed on the priority list for research on the other sex. This measure will help avoid discriminatory policies that can result from unequal data rather than unequal effects. Input should be sought from labor unions, community groups, and companies regarding the identification of agents or exposures that are of concern. These scientific evaluations should take into account: *a*) the quality of the data including experimental design, statistical power, and measurement of exposure and outcomes. These considerations apply to human, animal, and *in vitro* studies; *b*) for nonhuman studies, the validity of extrapolation to humans. Consideration should be given to knowledge of pharmacokinetics, site of action, and mechanism; *c*) the potency of the agent or the magnitude of the effect; *d*) the end point(s) affected; and *e*) the number of humans likely to be exposed. The evaluation given agents subject to Proposition 65 regulation in California should serve as a starting point for the development of an evaluation methodology (*13–15*).

A testing strategy should be developed to evaluate agents for reproductive and developmental toxicity before introduction into the workplace and general environment. Since the number of agents currently in use is quite large (greater than 60,000 by some estimates), agents in current use should be prioritized for testing. Criteria for prioritization should include the number of humans likely to be exposed, the likelihood that the agent is toxic (such as structural similarity to a known toxic agent), and its persistence or accumulation. In developing a testing strategy, the full range of testing techniques should be considered including computer analysis of structure–activity relationships, short-term *in vitro* assays, *in vivo* animal bioassays, and human epidemiologic studies.

While there are many gaps in our knowledge of reproductive and developmental risk, sufficient data currently exist to classify a number of agents as reproductive or developmental toxicants. Several lists of reproductive and developmental toxicants have been developed [e.g., the recent report on regulation of reproductive hazards by the General Accounting Office (*16*)]. This information should be acted upon immediately. Examples of actions to be taken include source reduction, regulatory activity, and/or risk communication.

The voluntary participation of companies, unions, and individuals is necessary for the conduct of human epidemiologic studies and should be encouraged. Policies should be formulated to encourage companies, unions, and industries to conduct and to participate in reproductive health research (both animal and human). It is also necessary to develop policies that will encourage individuals to participate in epidemiologic research. Low participation rates can seriously compromise the validity of epidemiologic research. Low participation rates are often a problem in studies requiring sampling of bodily fluids such as semen, blood, or urine. However, these studies show the greatest promise to elucidate exposure-effect relationships

because of their more precise measurement of exposure and/or health outcome. We therefore make the following recommendations to enhance participation in reproductive hazards research.

Researchers should adequately communicate study results to individuals, unions, and companies. It is very important for study subjects not to feel as if they have been used and discarded. Funds for notification should be built into the budget of all epidemiologic studies. Individuals should receive written notification of any test results that are clinically relevant as well as an explanation and interpretation of any unique or nonstandard tests that might be clinically relevant. This may include biological markers of subclinical effects. Individuals, companies, and unions should receive, in language they can understand, a summary of the overall results of the study.

Individuals, unions, and companies should be informed of the logistics of a research study and the goals of the study before it begins. Attempts should be made to involve them in the design of epidemiologic studies and the dissemination of results. Involvement in the design stage is appropriate only when it will not compromise the validity of the study. Individual participants should remain blind to specific study hypotheses only if this knowledge potentially affects their responses or the information gathered on them. Examples of involvement during the planning stages *may* include identification of health endpoints suspected to be in excess, designation of agents suspected to be toxic, identification of jobs with the greatest exposures, descriptions of work practices, and determination of appropriate means of communicating to employees or the community.

Investigators should disclose the source(s) of funding for research. Steps must be taken to ensure the confidentiality of data. Individual identifiable data should be protected from any form of disclosure including subpeona. Research on the factors that influence participation in epidemiologic studies should be encouraged. Attention should be directed to including neglected groups such as African Americans and Latino Americans in appropriate studies.

Teratogen Information Services should be encouraged to use a standardized reporting form and software to record exposure, gestational age at exposure (or pregestational time of exposure), outcome of pregnancy, and demographic information so that their experience can be more useful as research data. Data generated from these uniform reports should be collected, summarized, and published in hard copy and on diskette at regular intervals. This data might be used in the following ways: *a*) to generate hypotheses (i.e., sentinel health events and case series), *b*) to contribute to prioritizing agents for research (compounds or agents that generate the most inquiries), and *c*) to test hypotheses with prospectively collected data on individuals who call Teratogen Information Services. In particular, it may be possible to compare pregnancy outcome among women exposed to a particular substance with pregnancy outcome among women who were exposed to an innocuous substance. Nearly complete follow-up is essential as well as the control of potential confounders and the characterization of the population of individuals who use Teratogen Information Services.

Research Needs

There is a need for population-based data on reproductive and developmental endpoints. These data will allow us to determine the normal range and variability of these parameters. It will also allow us to examine geographic variation and secular trends. Such data are useful for surveillance, hypothesis generation, and hypothesis testing. Measurement of many of these parameters can be incorporated into existing periodic national surveys such as the National Health and Nutrition Examination Survey. Population-based data are needed on such endpoints as sexual behavior, semen characteristics, serum and urine endocrine parameters, sexual functioning, time to conception, and reproductive maturity and senescence.

There is a need for national statistics on such pregnancy outcomes as spontaneous abortion, congenital malformations, and birthweight. These data are necessary for surveillance as well as for hypothesis testing. This information could be compiled through a national template for birth certificates and registration of other pregnancy outcomes. Currently, each state has its own birth certificate and many do not require the reporting of spontaneous abortions. Definitions of stillbirth, spontaneous abortion, and congenital malformations also vary. A common uniform dataset should be established. Birth certificates should include a minimum set of information including last menstrual period; complications of pregnancy, labor and delivery; birthweight; Apgar scores; and congenital malformations. In addition, residential, occupational, demographic, and smoking information should be collected on the mother and the father. Spontaneous abortions and fetal deaths should be registered in the same fashion as livebirths regardless of gestational age at termination of the pregnancy. (Currently, most states require reporting only of terminations beyond 20 weeks). In addition, a mechanism should be developed whereby investigators can secure permission (after human subjects review) to contact individuals identified through vital records for further study. The state of Washington currently has such a mechanism in place.

There is a need for better occupational exposure data. Environments with multiple exposures are particularly difficult to assess. Major industries should be surveyed regularly to characterize actual exposures. This survey should include both questionnaire and industrial hygiene assessments of exposure. Better techniques need to be developed to quantify exposures, including further refinement of biological markers of exposure. The development and validation of biological markers of exposure should take into account issues such as pharmacokinetics and target organ dose.

There is a need to determine any significant risks of male-mediated developmental toxicity through both human epidemiologic studies and experimental animal studies. This includes an examination of such outcomes as spontaneous abortion, congenital malformations, low

birthweight, premature delivery, developmental delay, and childhood cancer.

There is a need to continue and expand work on the validation of the use of animal models in reproductive and developmental toxicity testing. The predictive value of a particular test or species may vary by class of compound. There is also a need to develop and to validate short-term *in vitro* assays for reproductive and developmental toxicity screening. This work will facilitate the evaluation of the large number of agents currently in use and introduced daily into our lives. These assays should also contribute to the elucidation of structure-activity relationships.

Techniques used to extrapolate from short-term or whole-animal toxicity testing to humans must be biologically based. Such research should include determination of pharmacokinetics, site of action, and the mechanism of action. Extrapolation and risk assessment will thereby be more valid and could include quantitative dose–response estimates. This information is also necessary to devise appropriate preventive/intervention strategies.

Functional effects in offspring (e.g., developmental or behavioral effects) should be included as end points in studies of reproductive and developmental toxicity. Functional effects, as well as structural or morphologic effects, should be included in epidemiologic studies. In addition, the predictive value of functional changes in animals needs to be further evaluated.

Reproductive health end points other than procreation should be included in studies of reproductive toxicity. Such endpoints include menstrual function, endocrine parameters, semen parameters, sexual function, and reproductive maturity and senescence. Physical factors should be evaluated for reproductive and developmental toxicity. These factors include temperature, light, noise, vibration, psychological stress, electromagnetic fields, and ergonomics.

There is a need to determine potential variation in susceptibility to the effects of reproductive or developmental toxicants. Susceptibility may vary with demographic, nutritional, or genetic factors, as well as health status and concomitant exposures. Very little is known about human susceptibility to reproductive toxicants or about synergistic effects of multiple exposures. Information on susceptibility is important because it has the potential to reduce the variability in dose–effect relationships (eliminating "noise"), thereby allowing more precise characterization of risk. Understanding those factors that mediate individual susceptibility may also lead to an understanding of the mechanism that leads to reproductive harm as well as the identification of specific components of complex mixtures that may be toxic. At the same time, it is important that information on susceptibility not be used to discriminate against workers or others.

Conclusions

No one should suffer reproductive harm from exposures at work or in the general environment. However, current scientific knowledge is often insufficient to predict those situations which will result in deleterious outcomes. In cases where adequate knowledge exists, regulatory action and voluntary control measures by industry should be implemented.

Responsible cooperation by labor, industry, government, individual citizens, and the scientific community is required to implement research needed to establish the scientific basis that will direct future reproductive policy decisions. Only with this cooperation can we expect to successfully implement a complex and demanding research agenda and prevent future reproductive harm.

Appendix

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