Exposure to Hazardous Substances and Male Reproductive Health: A Research Framework

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The discovery in the mid-1970s that occupational exposures to pesticides could diminish or destroy the fertility of workers sparked concern about the effects of hazardous substances on male reproductive health. More recently, there is evidence that sperm quantity and quality may have declined worldwide, that the incidence of testicular cancer has progressively increased in many countries, and that other disorders of the male reproductive tract such as hypospadias and cryptorchidism may have also increased. There is growing concern that occupational factors and environmental chemical exposures, including *in utero* and childhood exposures to compounds with estrogenic activity, may be correlated with these observed changes in male reproductive health and fertility. We review the evidence and methodologies that have contributed to our current understanding of environmental effects on male reproductive health and fertility and discuss the methodologic issues which confront investigators in this area. One of the greatest challenges confronting researchers in this area is assessing and comparing results from existing studies. We elaborate recommendations for future research. Researchers in the field of male reproductive health should continue working to prioritize hazardous substances; elucidate the magnitude of male reproductive health effects, particularly in the areas of testicular cancer, hypospadias, and cryptorchidism; develop biomarkers of exposure to reproductive toxins and of reproductive health effects for research and clinical use; foster collaborative interdisciplinary research; and recognize the importance of standardized laboratory methods and sample archiving. *Key words*: hazardous substances, male reproductive health, research, semen quality. *Environ Health Perspect* 108:803–813 (2000). [Online 21 July 2000]
http://ehpnet1.niehs.nih.gov/docs/2000/108p803-813moline/abstract.html

Paternal exposure to solvents, pesticides, and metals has been associated in animals and humans with the occurrence of spontaneous abortion, low birth weight, birth defects, childhood leukemia, brain cancer, change in the male:female sex ratio of offspring, and other end points related to growth and development. Certain paternal occupations—rubber worker, petroleum worker, agricultural chemical worker, painter, welder, and janitor—have been particularly implicated as detrimental to the reproductive health of men (*1*). The reproductive hazards of occupational exposure have been recognized by the National Institute for Occupational Safety and Health (NIOSH) as a priority area in need of further study. The National Occupational Research Agenda, coordinated by NIOSH, was established in 1996 to outline the research priorities that can lead to improved worker safety and health in 21 key areas of occupational health (*2*). One of the 21 priority research areas is fertility and pregnancy abnormalities, which includes male reproductive health.

However, exposure to environmental hazards is not limited to the workplace. Potential sources of exposure include food, air, water, soil, and hobbies. Individuals may have multiple exposures that in many cases occur chronically and at low doses. The reproductive health implications of chronic exposures to reproductive toxicants are not well documented and, in general, the mechanisms of toxicity are either poorly understood or unknown.

Reports of declining sperm counts over the past 50 years and other disturbing trends alerted scientists to the possibility that exposure to chemicals in the environment may damage male reproductive health. Testicular cancer, the most common malignancy in men 15–44 years of age (*3*), has increased markedly in incidence in this century in virtually all countries studied. The incidence of hypospadias, a developmental malformation of the male urethra, appears to be increasing worldwide. Cryptorchidism (undescended testicle), another developmental defect, may have increased in some human populations and appears to be increasing in wildlife (*4,5*).

The causes of these trends have not been identified and relevant toxicologic data about male reproductive effects of environmental toxicants are limited. Recent research efforts have focused on the possibility that exposures to hormonally active compounds, particularly during childhood and *in utero*, are to blame, at least in part, for changes in semen quality, increasing rates of testicular cancer, and malformations of the male urogenital tract. The ability to investigate environmental determinants of these indicators of male reproductive health is currently limited by available methodologies and data.

Male reproductive health is not measurable by any one variable. The male reproductive system is complex; its development is hinged on precisely timed events and full reproductive capacity is dependent on disparate physiologic processes. For example, an accurate picture of male reproductive capacity and function cannot be obtained solely through the measurement of a single sperm count. It is more correctly characterized by a variety of biologic markers, which together provide a more comprehensive picture than any outcome would on its own (*6*). In considering the state of male reproductive health, researchers must look broadly at relevant outcomes in addition to fertility, including disturbances in neuroendocrine hormone profiles, alterations in sexual functioning, the occurrence of cancers, and congenital defects of the male reproductive tract. As more information about the toxic effects

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This work was supported by NIH grant ES07198 (Superfund) from the NIEHS. Further support for the Hazardous Substances and Male Reproductive Health conference was provided by the U.S. Environmental Protection Agency, the National Institute for Occupational Safety and Health, the Agency for Toxic Substances and Disease Registry, and the New York Academy of Medicine.

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Received 24 January 2000; accepted 20 March 2000.

of hazardous substances emerges, male reproductive health may expand to include additional as yet unidentified variables.

Semen quality, in particular, should be considered in a more comprehensive manner. Research studies in the past have looked at sperm count (millions of sperm per milliliter of ejaculate) as the single measure of male reproductive health. Although sperm count is an important measure, there is a need to examine semen quality as a whole. Semen quality includes ejaculate volume, sperm motility, sperm morphology, functional variables, and tests of genetic integrity or damage. An important and emerging understanding in this field is that semen quality varies within and between individuals. There are seasonal variations in semen quality, and male fertility decreases somewhat with age. Abstinence preceding collection of a semen specimen plays a role in semen quality, with days of abstinence correlated to sperm count, sperm motility, and ejaculate volume. Subjective evaluation of some relevant outcomes, such as sperm morphology, limits precise comparisons of sperm quality. As automated systems are developed for measuring such outcomes, straightforward, reliable, and truly comparable data will be generated.

A comprehensive approach to exposure assessment is also important because men are exposed to complex combinations of potential reproductive toxicants and not simply to isolated hazards. Most exposures are multiple and overlapping. Moreover, chemicals might interact with the male reproductive system in different ways depending on other toxicants present in the body. Until methods to examine the effects of complex chemical mixtures are developed, research that examines one compound exclusively provides an incomplete picture of reproductive health effects.

On 14–15 May 1998, the National Institute of Environmental Health Sciences/Superfund Basic Research Program and the Mount Sinai School of Medicine convened a conference, Hazardous Substances and Male Reproductive Health, to develop strategies for understanding the importance of environmental effects on male reproductive health and fertility and to draft an international research agenda. Other sponsors of the conference were the U.S. Environmental Protection Agency (U.S. EPA), the National Institute for Occupational Safety and Health, the Agency for Toxic Substances and Disease Registry, and the New York Academy of Medicine. The research findings and recommendations arising from the conference provided a foundation on which the following framework for research was developed.

Ecoepidemiology: The Complementary Evidence of Reproductive Toxicity in Wildlife

Environmental pollutants have been linked to adverse male reproductive effects in wildlife species in classes from invertebrates to mammals (*7*). Although such reproductive outcomes have been studied most intensively in amphibians and reptiles, related male reproductive disorders occur in many species of wildlife. The causes of many of these disorders are unknown, but exposure to hormonally active agents in the environment is one possible explanation. The methods of ecoepidemiology address the challenges of evaluating the contributions of environmental pollutants to specific disease states in wildlife not living in controlled conditions (*8*). An ecoepidemiologic approach allows for the distinction between genetic causes (which may be enhanced by inbreeding in isolated populations resulting from isolation of subpopulations) and environmental causes of disease. Such an approach must begin with field evaluation, move into the laboratory, and then look at compounds on a mechanistic level.

The Lake Apopka alligator is one of the best-known wildlife species in which environmental xenoestrogens appear to have damaged the male reproductive system. The freshwater Lake Apopka in Florida is adjacent to the former Tower Chemical Company, which is now a Superfund site. From 1970 to 1980, a pesticide mixture containing high levels of DDT and its metabolites contaminated the area. Research comparing alligators from Lake Apopka to those in other Florida lakes found that Lake Apopka juvenile alligators have significantly smaller penis size as well as abnormalities in gonadal morphology and lower concentrations of plasma testosterone (*9*). Although the exact cause has not been determined, these alligators have significantly elevated serum concentrations of organochlorine pesticides compared to alligators at other lakes in Florida. The Lake Apopka alligators also have the breakdown product of DDT (DDE) stored in their body fat. Environmental xenoestrogens and antiandrogens such as these may contribute to abnormal or subnormal reproductive system development, and appear to act on the males specifically during embryonic development (*10,11*).

The Florida panther is another species in which environmental pollutants may be the cause of significant male reproductive effects (*11*). The endangered Florida panther suffers from inbreeding and vastly reduced habitat. The remaining population, estimated at only 30–50 individuals in 1995, exhibits a variety of problems, including reproductive and endocrine disorders (*11*). The males have an extremely high rate of cryptorchidism (estimated as 90% in 1995), as well as low ejaculate volume; low sperm concentration; poor sperm motility; and high proportion of sperm with morphologic abnormalities. Because of the small population size and the reduced habitat area, researchers have hypothesized that inbreeding may be the cause of the myriad health problems, but another possible explanation is exposure to environmental xenoestrogens. Florida panthers eat hogs, deer, armadillos, and raccoons. Raccoons are suspected to account for much of the exposure to environmental xenoestrogens. In that area raccoons, which eat fish, bioaccumulate mercury as well as endocrine-disrupting pesticides (*12,13*). Although the causes of the reproductive problems in the Florida panther have not been identified, estrogen mimics and antiandrogens in the environment are emerging as a strong possibility. This has significant implications for other wildlife species as well as for humans.

Male reproductive problems in wildlife provide a complementary picture to human health effects. Such trends are helpful in evaluating environmental threats to human reproductive health, and have enabled researchers to focus on substances that, because of their effects on wildlife, are likely to pose reproductive health hazards in men.

Evidence of Environmental Impact on Male Reproductive Health in Humans

During the past decade, significant attention has focused on reported trends of declining male reproductive health. Almost all cancer registries in the Western world have noted remarkable increases in testicular cancer incidence (*3*). Several indications of decreasing semen quality have been noted (*14–18*). There are also data to suggest that the incidence of certain urogenital abnormalities, including hypospadias and cryptorchidism, have become more common (*19*). The fact that these reported changes in male reproductive health occurred concurrently within a relatively short period of time suggests that common environmental factors may be of importance. Normal sexual differentiation, normal development of the gonads, and normal postnatal development are essential for normal reproductive function in adulthood; therefore, it has been suggested that a common fetal factor could play a role in all of the observed trends (*19*). Based on epidemiologic evidence from studies of children of mothers who were exposed to diethylstilbestrol in early pregnancy (*20,21*) and experimental evidence from the administration of synthetic

estrogens to pregnant animals (*22–26*), it is hypothesized that hormonally active agents, which are ubiquitously distributed in the environment, could play an etiologic role. Researchers from several countries, including Denmark, the United Kingdom, and the United States, have published reports that delineate the current state of knowledge and provide suggestions for further research to address these hypotheses (*19*).

Testicular cancer. Testicular cancer is the most common malignancy among men 15–44 years of age, with a peak incidence between 18 and 35 years of age (*27,28*). Environmental influences are likely to play an important causal role in this disease, which has shown marked geographic variation (*29*). Testicular cancer incidence is highest in Denmark (*30,31*), Switzerland (*32*), and New Zealand (*33*), with incidence rates of up to 8 per 100,000 per year, and the evidence is overwhelming that testicular cancer incidence has increased rapidly in virtually all countries studied (*3*).

The increases in incidence cannot be attributed to improvements in diagnosis or surveillance because the observed increases are too consistent and too large and because the principal means of diagnosis has been and remains the detection of a testicular mass on direct physical examination. In the United States, for example, the testicular cancer incidence rate among white active-duty servicemen 17–44 years of age increased by 61% from the 1970s to the 1990s (*34*). This increase in incidence was most striking for those 30–-34 years of age, in whom testicular cancer rates doubled during a time in which there was no change in procedures for diagnostic detection. The rising incidence is particularly disturbing given the otherwise careful physical screening and good health of this young adult male population.

Increases in the incidence of testicular cancer have not been uniformly reported among all men. The incidence of testicular cancer in Nigeria, for example, is 0.1 per 100,000 per year (*35*). African Americans in the United States also have had consistently low incidence over time. Between 1973 and 1996, for example, rates of testicular cancer increased 51.2% in white men and the rates for black men increased 17.3% (*36*). Although genetics almost certainly plays a major role in the etiology of the disease, other etiologies, including environmental factors, need to be elucidated to explain why, for example, major differences in testicular cancer rates exist among the relatively genetically homogenous Scandinavian countries.

Increases in testicular cancer rates are not recent phenomena. A doubling in incidence was documented in Denmark within 25 years after the initiation of cancer registration in 1943 (*3*). Mortality data from Great Britain show an increase in mortality due to testicular cancer beginning in the 1920s (*37*). These mortality data raise an important distinction: if environmental risk factors play a role in testicular cancer incidence, relevant exposures must therefore have existed since the turn of the century. This would make it less likely that organochlorines such as DDT and other endocrine-disrupting chemicals are possible etiologic agents.

Because testicular cancer occurs in young adults, major etiologic factors may operate early in life, perhaps even *in utero* (*3*). Trends in testicular cancer suggest that lifetime exposure to environmental risk factors appears more strongly related to birth cohort than to year of diagnosis (*3*), implying that early exposures may be most relevant for the development of cancer. Such an observation has important implications for prevention. Efforts would have to be aimed toward women and men of childbearing age, pregnant women, and neonates. Possible etiologic agents for testicular cancer include abnormal sex hormone exposure related to endocrine disruptors in the environment, maternal parity (*38*) and age (*39*), high or low birth weight (*40*), age at puberty (*41*), use of the pesticides atrazine (*42*) and *N,N*-diethyl-*m*-toluamide (*43*), and exposure to workplace hydrocarbon (*44*) and polyvinyl chloride (*45*).

Testicular cancer disproportionately affects men with undescended testis (cryptorchidism), Klinefelter syndrome, hypospadias, and infertility. The risk of developing testicular cancer rises dramatically in men with disorders of sexual gonadal development (*46*). These include mixed gonadal dysgenesis, androgen insensitivity, and male pseudohermaphrodism (*47*). Nearly all of these conditions are also characterized by delayed differentiation of the testicle and infertility.

Epidemiologic studies have reported the relative risk of testicular cancer in men with cryptorchidism as 3–14 times the normal expected incidence (*39*). In men with unilateral cryptorchidism, the contralateral normally descended testicle is also at an increased risk of developing testicular cancer. Between 5 and 10% of men with unilateral cryptorchidism develop testicular cancer in the contralateral testicle and in nearly 50% of men with bilateral testicular cancer, a history of cryptorchidism is present. This observation is consistent with the hypothesis that the inciting events resulting in cryptorchidism have a negative impact on the normal development of both testes (*47*).

Testicular cancer patients have much poorer semen quality than other cancer patients, and a recent epidemiologic study shows that men who have testicular cancer are subfertile even before they develop clinically detectable cancer (*48*). This suggests causal factors shared by both subfertility and testicular cancer (*49*). Research is ongoing to explore new genetic markers for early detection of carcinoma *in situ* cells in semen, as well as to define the role of hormonal assays (e.g., inhibin-B) as screening tools for testicular cancer and carcinoma *in situ*.

Hypospadias and cryptorchidism. Two male genital birth defects, hypospadias and cryptorchidism, both apparently representing mild degrees of feminization, have become important in the ongoing debate regarding the significance of endocrine disruptors or other environmental influences on male development (*50*). Several researchers have reported increases in each of these defects in the past three decades (*4,51*). To evaluate the hypothesis of common etiologies, pre- and perinatal determinants of hypospadias, cryptorchidism, testicular cancer, and infertility are under investigation. Abnormal sex hormone exposure during critical periods of development has been postulated as a likely shared pathologic mechanism (*19*).

Hypospadias is a developmental malformation in which the urethra opens on the underside of the penis or on the perineum. If untreated, hypospadias can lead to urinary stricture, infection, and difficulties with ejaculation. Reports of increasing rates of hypospadias during the 1960s, 1970s, and 1980s have raised concerns about the dependability of surveillance systems, especially regarding inconsistencies in diagnosis due to a classification scheme that depends on the distance of the urethra opening to the tip of the penis. Hypospadias, particularly in the mildest degree, may be incorrectly classified by clinicians. Despite the difficulties of using surveillance system data, however, it appears that rates of hypospadias are increasing worldwide. The increasing rates do not solely reflect improvements in reporting and diagnosis, because there are increases for severe hypospadias as well as for mild cases.

Data from a large number of countries address the questions of whether increases in hypospadias are continuing and whether the direction of trend lines is correlated with increasing industrialization. The International Clearinghouse of Birth Defects Monitoring Systems (Rome, Italy), which collects data from 29 countries on five continents, has assembled data that form the basis of an analysis of global trends in hypospadias. The incidence of hypospadias does not appear to be associated with industrialization (as measured by gross domestic product), although there has been an increase in reported hypospadias rates in recent decades in the

majority of international surveillance systems. Hypospadias rates increased in 18 of 29 systems (62%) and declined in 11 systems, although the increases may have slowed or stopped since 1985. Whereas improved reporting and diagnosis cannot account for the increases, possible causes of the upward trend in hypospadias rates include demographic changes and endocrine disruption, among others (*52*).

Cryptorchidism, another male developmental defect, is characterized by the failure of one or both testicles to descend into the scrotum. Cryptorchidism is a well-established risk factor for subfertility and testicular cancer, strongly suggesting a common etiology affecting germ cell development. Because the defect resolves spontaneously by the first birthday in > 70% of affected infants, there are inconsistencies in its diagnosis. If the condition is diagnosed before the first birthday, or if gestational age is miscalculated in premature babies, there may be an overestimation of the incidence rate. Although the incidence of cryptorchidism does not appear to be increasing worldwide in humans, data on this defect are limited, and two U.S. surveillance systems have shown marked increases (*52*). The defect does appear to be rising in wildlife populations such as the Florida panther (*11*), and demasculinization and feminization have been linked in other wildlife populations to environmental exposure to endocrine disruptors (*7*).

Semen quality. Reports suggesting that sperm counts have declined in certain areas of industrialized countries throughout the world (*14,16–18,53–56*) have contributed to concern about a possible worldwide decline in human semen quality. A meta-analysis by Carlsen et al. (*14*) in 1992 reported a worldwide decline in sperm counts over the preceding 50 years, concluding that mean sperm concentrations had decreased by almost 50% from 1940 to 1990. A 1995 study reported a 30% decrease in sperm concentration in Paris over a 20-year period among fertile sperm donors from a single sperm bank (*53*). Numerous researchers have attempted to determine whether this apparent decline is real or due to unrecognized biases in data collection and analysis (*18,57*).

Confounding variables may account for the observed findings. Potential confounders include increasing donor age, duration of abstinence, frequency of ejaculation, and even the season of sample collection, all of which influence sperm variables. Other suggested confounders include smoking, radiation exposure, stress, ethnicity, and a variety of physical conditions including varicocele, infection, and genital abnormalities such as hypospadias and cryptorchidism. In addition, differences in methodology used to perform the semen analysis may produce inaccuracies. For example, there is interobserver variability when comparing sperm counts from different databanks, and there are measurement inaccuracies of up to 30% depending on the counting chamber (*58*).

In a 1996 paper, Fisch et al. (*58*) examined geographic variability as a potential confounding variable that may significantly affect reported temporal trends in sperm counts. The authors observed that sperm count within the United States depended on geographic location, with the highest counts occurring in New York City samples, and suggested that the observed differences in semen quality may simply reflect the clustering of significant geographic determinants. Theories explaining the apparent geographic disparities in sperm counts are currently only speculative, and include environmental, socioeconomic, racial, and methodologic differences (*18,59*). Fisch et al. (*58*) reported yearly fluctuations in mean sperm counts and birth rates (*60*), suggesting that this may be a more important variable than previously considered.

Methods of Assessing Male Reproductive Capacity

Toxicants can affect the male reproductive system at one of several sites or at multiple sites. These sites include the testes, the accessory sex glands, and the central nervous system, including the neuroendocrine system. There is no single all-encompassing marker of reproductive capacity in men, and there is no consensus among researchers about what constitutes an appropriate battery of validated and interpretable variables of male reproductive function for use in research and clinical settings. We review the state of the science and the methodologies with greatest promise from three areas—experimental toxicology, epidemiology, and chromosomal or genetic toxicity.

The contribution of experimental models. The usefulness of experimental animal models is usually perceived as limited to hazard identification using the test protocols specified by regulatory agencies. However, animal models can also provide valuable support to reproductive risk assessment on many other fronts. If the focus is on human exposure, animal studies can be designed to confirm reproductive toxicity when initial observations in exposed humans are suggestive of an adverse effect. Furthermore, such observations can be extended across a wide range of exposures in animals, using any route of exposure and any specified dose versus time scenario. For example, when human exposure is likely to be acute or intermittent, animal models are ideal for defining critical exposure windows based on developmental stage or for revealing the pathogenesis of an effect at various times after exposure through recovery. This is particularly important with respect to male reproductive effects because alterations in semen quality or fertility may not become evident until some time after the exposure, particularly if an early stage of spermatogenesis is targeted.

A rodent model is most commonly used for the study of reproductive and developmental toxicity (*61*). To use toxicology data derived from animal studies to advantage in risk assessment, it is critical to identify and understand species-specific differences in physiology and metabolism that may affect the response to the toxicant in question. It is also important to recognize that the genetic homogeneity of rodents, although advantageous in its lack of potential confounding factors, makes it difficult to study susceptible subpopulations unless different strains are studied. Nevertheless, rodent studies provide valuable information about hazard identification, dose response, and critical thresholds for fertility, and are often helpful for developing paradigms for human studies. Rodent models have, for example, been used to determine the relationship between sperm end points and function (fertility) (*62*).

Determining that a substance is toxic to the male reproductive system is only the first step: The next step is to examine its mechanisms of toxicity. Mechanistic information allows for predictions about the potential toxicity of individual compounds or complex mixtures in humans, for better understanding of the windows of vulnerability in the development of the male reproductive system, and for developments of possible preventive or curative measures.

Acute short-term exposure models combined with serial exposure models give a complete picture of the range of effects (*61*). Exposing animals over a long period of time allows for the detection of transgenerational effects from chemicals, such as male-mediated developmental effects. If developmental effects appear, researchers can go back and administer a dose during that critical period of development to refine knowledge about how such problems occur. Early developmental end points measurable in animal research include anogenital distance at birth, testis position, genital malformations, secondary sex characteristics, and serum hormone levels. Acute short-term exposures, on the other hand, can be useful for identifying critical windows of exposure. Acute exposures followed over time can help identify the pathogenesis of a lesion, isolate the cell type that is susceptible to damage (germ cells, spermatocytes, or spermatid), and determine genetic effects, including the repair capability

of affected genes. Serial sacrifice studies are best used for identifying the earliest detectable pathologic changes in target organs, cells, or processes. Multigeneration studies, in particular continuous breeding studies, yield the most thorough assessment of the many complex processes that result in reproductive and developmental toxicity.

Epidemiologic approaches. Epidemiologic methods for assessing the impact of hazardous substances on male reproductive health include *a*) questionnaires to determine reproductive history and sexual function, *b*) reproductive hormone profiles, and *c*) semen analysis. The choice of appropriate methodologies to study the effects of reproductive toxicants is predicated on the investigators' understanding of several factors: the nature of the exposed population; the source, the levels, and the known routes of exposure; the organ systems in which a toxicant exerts its actions; the hypothesized mechanisms of a toxicant's actions; and the techniques available to assess the effects of toxicants in the relevant organ systems (*63,64*). Table 1 outlines the methods currently available for assessing the principal targets of male reproductive toxicants in humans—the testes, the accessory sex glands, the neuroendocrine system, and sexual function. Researchers and clinicians interested in male reproductive health and fertility are using increasingly sophisticated methods adapted from the fields of assisted reproductive technology and reproductive toxicology, including assays of sperm function, genetic integrity, and biomarkers of DNA damage. For population-based studies involving occupational groups or communities with environmental exposures, issues related to the cost, validity, precision, and utility of these methods must be carefully considered.

The testis, the site of sperm cell production and the target organ for genetic damage, is most often studied. Occupational exposures to lead, dibromochloropropane, ethylene dibromide, and glycol ethers affect sperm production in humans (*1,65–68*). To establish the extent of toxicity to the testis, researchers can measure the size of the testis, obtain a semen sample, or take a testicular biopsy. Standard semen analyses (including semen volume, sperm concentration, total sperm count, motility, and morphology) have been the primary research tools for studying the effects of toxicants on the male reproductive system. Epidemiologic studies have successfully utilized semen quality as a marker of fertility (*60,69*) although not without problems (*70–74*) (e.g., potential selection bias due to low compliance rates and substantial intraindividual variability in semen variables resulting in misclassification based on the static results of a single analysis). In contrast to longitudinal studies as well as clinical evaluations where more than one semen sample is required (*75*), research has shown that in cross-sectional epidemiologic investigations, a single semen sample from each participant generally is sufficient if obtained under defined conditions and according to a set protocol (*72,73,76*). Methodologic questions regarding intraindividual variation and the precision and reliability of assessment techniques can be addressed to some extent. Individual semen samples can be split and replicate measurements made. The mean value from multiple aliquots can be used and intraclass correlation and coefficients of variation can be determined. Individualized contact and follow up with the study subjects, the provision of financial incentives, and the use of mail-in containers that allow men to collect the semen sample at home are factors that may increase response rates in epidemiologic studies (*77*).

As much as any other factor, uncertainty in the results of studies addressing threats to male reproductive health stems from debate about the definition of normal semen quality and whether or not expected fluctuations are distinguishable from diminished reproductive capacity resulting from hazardous exposures (*72,73,78,79*). In epidemiology studies conducted to investigate effects of an accidental exposure, it may be difficult to enroll an appropriate unexposed control group. In such cases, results obtained from exposed populations can be interpreted with respect to the reference values established for routine semen measures by the World Health Organization methods (*80*) and other well established criteria (*81*).

It has proved more difficult, however, to resolve questions about the validity of using semen measures to assess human fertility (*59,69,82*). Which semen variables are the most sensitive with respect to perturbation by toxicant exposure, and which are the most predictive of human fertility? Can threshold levels associated with impaired fertility be defined? Are shifts in sperm quantity and quality within populations related to measurable decreases in normal live births? Some relevant information about these questions has been provided by animal models (*62*). However, because these models rely on observations of the group as a whole, they have not been as useful in elucidating intraindividual variability, which impedes our ability to apply results from these models to humans. The uncertainties associated with traditional semen measures have led to the recent development of assays of sperm function and genetic integrity; these assays may prove more sensitive and more specific reflections of toxicant-induced effects (e.g., aneuploidy or reduced sperm motility) in individuals (*76,83*).

Although any of the commonly used epidemiologic study designs can be used to study male reproductive health (e.g., case–control studies of occupational risk factors for congenital defects or other adverse pregnancy outcomes and retrospective cohort studies for cancers of the genitourinary tract), the most promising current and future initiatives use prospective cohort studies that are designed to test specific exposure-outcome hypotheses (*84*). Currently, several ongoing studies are addressing relationships among measures of semen quality, fertility, pregnancy outcomes, and exposures. These investigations have been designed to take into account potential confounders such as geographic variability; factors related to the female partner including abnormal menstrual cycle, previous treatment for infertility,

Table 1. Assessment of male reproductive capacity in humans.

Method of assessment	Endocrine system	Testes	Posttesticular events[a]	Sexual function
Luteinizing hormone		–	–	–
Prolactin		–	–	–
Testosterone		–	–	–
Inhibin-B		–	–	–
Sperm density	–		–	–
Sperm morphology and morphometry	–			–
Sperm motility (% motile and velocity)	–			–
Sperm viability (vital stain and HOS)	–	–		–
Semen volume	–	–		–
Semen pH	–	–		–
Marker chemicals from accessory glands	–	–		–
Sperm function assays[b]	–			–
Sperm genetic analyses[c]	–			–
Nocturnal penile measurements	–	–	–	
Personal reproductive history[d]			–	

Adapted from Schrader (*67*) and Schrader and Kesner (*86*). HOS, hyperosmotic swelling.
[a]Includes production of seminal plasma components by sex accessory glands and maturation of sperm in the epididymis. [b]Includes acrosome reaction, hemizona assay of sperm binding, and sperm penetration assays. [c]Includes sperm chromatin stability assay, Comet assay, and assessment of chromosomal aneuploidy and nuclear microdeletions. [d]Includes pubertal development, paternity (pregnancy timing and outcomes), sexual function (erection, ejaculation, orgasm, and libido).

and parity; contraception status; and abstinence interval (*85*).

The accessory sex glands, which include the epididymis, prostate, and seminal vesicle, may also be targets of toxicants (*67*). Ethylene dibromide is one substance that affects the accessory sex glands after occupational exposure (*68*). Alterations in sperm viability, as measured by eosin stain exclusion or by hypoosmotic swelling (*86*) or alterations in sperm motility variables (*72*), suggest a problem with the accessory sex glands. Biochemical analysis of seminal plasma provides insights into glandular function by evaluating marker chemicals secreted by each respective gland (*67*). For example, the epididymis is represented by glycerylphosphorylcholine, the seminal vesicles by fructose, and the prostate gland by zinc. Measures of semen pH and volume provide additional general information on the nature of seminal plasma, reflecting posttesticular effects. A toxicant or its metabolite may act directly on accessory sex glands to alter the quantity or quality of their secretions. Alternatively, the toxicant may enter the seminal plasma and affect the sperm or may be carried to the site of fertilization by the sperm and affect the ova or conceptus. The presence of toxicants or their metabolites in seminal plasma can be analyzed using atomic absorption spectrophotometry or gas chromotography/mass spectrometry.

Impact on the neuroendocrine system is another mechanism whereby toxicants can disturb the male reproductive system. Lead, stilbene, and kepone affect the endocrine system in occupationally exposed men (*87*). To establish the extent of endocrine dysfunction, hormone levels can be measured in blood and urine. The profile recommended by NIOSH to evaluate endocrine dysfunction associated with reproductive toxicity consists of assessing serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and prolactin (*67*). Because of the pulsatile secretion of LH, testosterone, and to a much lesser extent FSH, and the variability in the evaluation of reproductive hormones, it is recommended that three blood samples be drawn at set intervals in the early morning and the results pooled or averaged for clinical assessment (*88,89*). In epidemiologic field studies, however, multiple blood samples are impractical and may decrease participation rates (*90*). Alternatively, LH and FSH can be measured in urine, providing indices of gonadotropin levels that are relatively unaffected by pulsatile secretion. However, if an exposure can affect hepatic metabolism of sex steroid hormones (*91*), urinary measures of excreted testosterone metabolite (androsterone) or estradiol metabolite (estrone-3-glucuronide) are not recommended.

Future assessment of reproductive hormones may extend to inhibin, activin, and follistatin, polypeptides that are secreted primarily by the gonads and that act on the pituitary to increase (activin) or decrease (inhibin and follistatin) FSH synthesis and secretion. Within the gonads, these peptides regulate steroid hormone synthesis and may also directly affect spermatogenesis. Ongoing studies are investigating the utility of serum inhibin-B level as an important marker of Sertoli cell function and *in utero* developmental toxicity (*92,93*).

Other indicators of central nervous system toxicity are reported alterations in sexual function, including libido, erection, and ejaculation. There is not much literature on occupational exposures causing sexual dysfunction in men (*67*); however, there are suggestions that lead, carbon disulfide, stilbene, and cadmium can affect sexual function. These outcomes are difficult to measure because of the absence of objective measures and because sexual dysfunction can be attributed to and affected by psychologic or physiologic factors (*67*).

Biomarkers of genetic damage. Biomarkers of chromosomal and genetic damage are increasingly used in the search to understand abnormal reproductive health outcomes, in part because of the possibility that there may be identifiable genetic polymorphisms which make an individual more susceptible to the adverse reproductive effects from exogenous substances. These assays provide promising and sensitive approaches for investigating germinal and potentially heritable effects of exposures to agents and for confirming epidemiologic observations on smaller numbers of individuals. Efficient technology for examining chromosomal abnormalities in sperm has only been developed recently.

Chromosomal abnormalities are primarily of two types: numerical and structural. Both kinds can be attributed in some cases to paternal factors. Karyotype studies have shown that although oocytes demonstrate a higher frequency of numerical chromosomal abnormalities, human sperm demonstrate a higher frequency of structural abnormalities with less frequent numerical abnormalities (*94*). In assessing sperm exposure to toxicants, it is therefore imperative to assess DNA structural integrity and not just chromosomal count.

Aneuploidy is a chromosomal abnormality that causes pregnancy loss, perinatal death, congenital defects, and mental retardation. Aneuploidy, a disorder of chromosome count, is observed in approximately 1 in 300 newborns (*95*). It is speculated that of all species, humans experience the highest frequency of aneuploidy at conception, with estimates ranging from 20 to 50% (*95*). Spontaneous abortions occur in at least 10–15% of all clinically recognized pregnancies. Of these, 35% contain chromosomal aneuploidy. Despite such a high frequency, there is little information about what causes this abnormality in humans.

Paternal origins of aneuploidy and other genetic abnormalities can be analyzed by studying chromosome complements in human sperm. Two types of analyses provide data on chromosomal abnormalities in human sperm: sperm karyotype analysis and fluorescence *in situ* hybridization (FISH) (*94*). Each technique has advantages and disadvantages. Sperm karyotyping is performed after sperm have fused with hamster oocytes. It provides precise information on numerical and gross structural abnormalities of all chromosomes from a given spermatozoon. However, only a limited number of sperm can be evaluated in each assay, and only those sperm that fertilize the oocytes are analyzable. Furthermore, this assay is technically difficult, labor intensive, expensive, and requires the use of animals (*96*). Also, it is better suited for clinical than for field studies because it must be performed on fresh semen. FISH, on the other hand, relies on the use of chromosome-specific probes to detect extra chromosomes (aneuploidy) or chromosome breaks or rearrangements in sperm. It is performed directly on sperm cells, eliminating the need for the use of animals. Although information is gained only for several chromosomes at a time, slides can be reprobed to increase the number of chromosomes evaluated. Furthermore, FISH can be conducted on archived sperm (either frozen or dried on slides), making it ideal for use in field studies. However, because the incidence of sperm aneuploidy is low, many cells (up to 10,000 per semen sample) must be evaluated, which requires significant scoring times. In comparison to karyotype analysis, however, FISH is relatively inexpensive and technically simpler, and data are obtained on all sperm, not just the ones that are capable of fertilization. These two techniques complement each other, with FISH providing information on large numbers of cells and karyotyping providing more precise and detailed information (*94,97–99*).

Several risk factors have been identified for increasing sperm chromosomal abnormalities. A sperm karyotype study of radiotherapy patients before and after treatment demonstrated a significant increase in the frequency of both numerical and structural chromosomal abnormalities up to 3 years after treatment (*83*). Assessing aneuploidy rates using FISH in men who received chemotherapy has yielded varying results based on agent, dose, timing of specimen collection, and type of cancer studied. Male

infertility is also associated with an increased frequency of chromosomally abnormal sperm karyotypes (*94*). These findings are of concern because infertile men may be candidates for intracytoplasmic sperm injection (ICSI), and therefore may be at increased risk of having children with chromosomal abnormalities.

Paternal age is another risk factor (*96*). Although increasing maternal age is irrefutably associated with an increased risk of nondisjunction in the oocyte, the relationship between paternal age and the frequency of nondisjunction has not been clearly elucidated. The father's influence on the fetus, however, may begin long before conception. Fetal development may be affected if the male has been exposed to lifestyle and/or occupational hazards. For example, recent studies show that smoking and/or alcohol consumption are associated with an increased risk of sperm aneuploidy (*100,101*). Multivariate analyses of national data by the March of Dimes suggest that paternal factors such as age or education may play an important role in birth outcomes such as low birth rate and infant mortality (*102*).

Preliminary data from a study sponsored by the Czech Ministry of the Environment and the U.S. EPA (*103*) demonstrated a significant association between exposure to air pollutants and aneuploidy in sperm. FISH was used to detect chromosomes X, Y, and 8 in spermatozoa of nonsmoking men 18 years of age. Men exposed to the heaviest air pollution had an elevated level of sperm with an extra Y chromosome (YY8), although other disomies were not increased (*103*).

These examples illustrate how genetic markers are starting to be used in the evaluation of male reproductive health to highlight subtle but important effects of environmental risk factors.

Recommendations for the Future

Despite extensive research into environmental influences on male reproductive health, the scope of the problem is still unknown. Standardized epidemiologic investigations, including collaborative international studies of reproductive health parameters in general populations, may help to improve knowledge; however, defining optimal strategies for collecting relevant data presents a significant challenge for researchers and clinicians working in all areas of male reproductive health.

The determinants of the various end points of male reproductive health and dysfunction—including infertility, sexual dysfunction, sperm characteristics, and birth outcomes—are poorly understood. There is abundant evidence that the male reproductive system can be influenced by exposure to hazardous substances during development, requiring the incorporation of preconception periods in both human and *in vivo* experimental animal studies. However, there is also limited information about the mechanisms and the impact on male reproductive function of specific environmental exposures. It is therefore important that clinical researchers and epidemiologists work with basic scientists to strategize about what substances to focus on and how exposures to these substances can be prevented.

The five recommendations for the future assume that a vital collaboration across disciplines and between government and academic institutions will be the most efficient and effective way to fill in the many gaps in knowledge. Moreover, collaborative international research will increase the capacity of researchers to make reliable comparisons of study populations.

Prioritize hazardous substances. Limited information is available about the relationship between environmental exposures and reproductive risk in humans. Most of our knowledge on this subject comes from studies of occupational exposures. To design studies and collect data that will have the greatest potential to identify and characterize reproductive risks to humans, it is important to prioritize known or suspected reproductive toxicants.

The evaluation of chronic, intermittent, and multiple exposures poses some of the most significant problems in all areas of environmental research. These kinds of exposures are difficult to specify and quantify, and the data needed to elucidate mechanisms of action for many chemicals and physical agents are often absent or inadequate. Although short-term exposure to environmental contaminants may cause dysfunction in the maturation process of the spermatozoa, for example, long-term exposures may disrupt neuroendocrine function, making it difficult to determine the initial cellular target and mode of causality in adverse male reproductive health outcomes. Assessing cumulative risks of exposure to multiple toxicants is more difficult than evaluating chemicals individually. Methods to examine the effects of complex chemical mixtures to protect human health in the real world must be developed. There are gaps in our understanding of the risks of chronic exposure to low levels of toxicants such as pesticides.

The U.S. EPA is required to evaluate reproductive risks of exposures to pesticides and other toxic substances, as well as to develop tests to screen for chemicals with potential to disrupt the endocrine system. The U.S. EPA multigeneration reproductive toxicity test was recently modified (1998) to add more specific indicators of reproductive organ function such as sperm measures (*104*). The revised test is better suited to detect more subtle effects on spermatogenesis and sperm maturation that might not be of sufficient magnitude to alter fertility in rodents but could be relevant to assessing risks in humans. The revised test also includes evaluation of sexual differentiation and maturation and is therefore better able to detect environmental endocrine disruptors (*104*). This test and similar tests used by the U.S. National Toxicology Program are suitable for hazard identification and are therefore important sources of information for prioritizing chemicals for further research including determination of the mode of action and cellular/molecular mechanisms of action.

The U.S. EPA reproductive toxicity tests and harmonized protocols recommended for use by the Organisation for Economic Cooperation and Development (*104,105*) are being applied voluntarily by industry to the large group of high-production volume (HPV) chemicals for which reproductive toxicity data are often lacking. HPVs, which number more than 3,000, are defined as chemicals produced at a rate of more than one million pounds per year. A recent evaluation of U.S. EPA chemical data showed that nearly half (43%) of these HPVs have not been screened adequately for toxicity (*106*). Therefore, the U.S. EPA, albeit a valuable source of toxicity data, cannot be the only source of information about priority substances. Researchers and other government agencies must endeavor to define and explore other potential hazards.

Evidence of reproductive effects derived from observations and studies in wildlife species may provide additional valuable clues about risks in humans despite interspecies differences among reproductive systems. Thus all sources of toxicologic information, whether obtained in humans, test species, or wildlife species, should be considered when prioritizing chemicals for further research.

Elucidate the magnitude of male reproductive health effects. Testicular cancer. The rising incidence rate of testicular cancer among young men seems to indicate that new risk factors have arisen or that influences from previous factors have intensified. Because testicular cancer occurs in young men, any hypothesis about testicular carcinogenesis must consider major etiologic factors that may operate before conception, *in utero*, and early in life. New analytical studies are needed to further assess risk factors for this cancer, including assessment of early infections, low fertility and infertility, occupational exposures, dietary factors, and early exposure to abnormal levels of exogenous or endogenous hormones.

It is important that researchers and clinicians assess the incidence and increase awareness of the risk of testicular cancer and carcinoma *in situ* in infertile men. Furthermore, it is essential to establish which of the available screening tools (e.g., manual testicular exams, scrotal ultrasonography, and cytogenetic assays) are feasible and effective for early detection of testicular cancer and carcinoma *in situ*.

Hypospadias and cryptorchidism. More standardized diagnostic criteria must be developed so that hypospadias and cryptorchidism can be uniformly recognized and enumerated around the world. This will allow researchers to move toward an understanding of the causes of these disorders. For their investigation of male reproductive health, researchers depend not only on laboratory results but also on clinical diagnoses. It is critical that diagnostic procedures and classification be comparable around the world. For example, cryptorchidism, which resolves spontaneously in 70% of affected infants by their first birthday, should not be included in surveillance systems until the first birthday. Moreover, clinicians should be made aware of the importance of developmental age when evaluating infants born prematurely because it will affect the morphology of the testes. Researchers should also continue to identify and use existing reliable data sources and develop additional surveillance systems for tracking the incidence of these anomalies temporally and geographically.

Clinical applications. Urologists, reproductive endocrinologists, and other clinicians can play a significant role in advancing biomedical research and prevention of adverse reproductive outcomes. They are on the front lines in the evaluation and treatment of male reproductive dysfunction; therefore they can also play a significant role in the collection of data important for studying male reproductive health.

Clinicians should incorporate assessment of environmental risk factors into their examination protocol when they evaluate infertile couples or treat men with reproductive dysfunction or diseases (*75*). When men present with sexual dysfunction, for example, clinicians can develop objective means of differentiating the influences of environmental factors on sexual functioning from the influences of psychologic or physiologic factors. Currently, methods for such differentiation do exist; for example, by monitoring the frequency and quality of nocturnal erections.

When exposure to an environmental toxicant is suspected, clinicians should initiate a physical examination including assessment of testicular size and abnormalities; a semen analysis; a hormonal profile; measurements of specific toxicants; and, in extreme cases, a testicular biopsy. Before the initiation of assisted reproductive techniques such as *in vitro* fertilization or ICSI, the man should have a complete work-up, involving, as needed, a urologist, andrologist, geneticist, and occupational medicine physician, to investigate the etiology of the dysfunction or disease.

Clinicians should also initiate prevention or minimization of known hazards by counseling patients to avoid exposure, to protect themselves during exposure, and to seek treatment after exposure. Clinicians can be the driving force behind public education on known reproductive hazards.

Because of the clinicians' critical role, it is essential that medical schools devote more teaching time to environmental health issues and make students aware of the growing specialty of environmental and occupational medicine (*107*).

Clinicians also need accurate information about environmental toxicants available in an efficient format. A centralized source of reliable information needs to be established and publicized for physicians who treat male reproductive dysfunction. An example of this type of resource has recently been developed for pediatricians, obstetricians, and other family care providers. *The Handbook of Environmental Health for Children* (*108*) is a comprehensive review of known environmental hazards affecting children, as well as guidance for evaluating, treating, and preventing these exposures.

Develop biomarkers of exposures and male reproductive health for research and clinical use. Resources must be invested in developing more advanced biomarkers of exposure to reproductive toxicants and of reproductive health outcomes. Advanced biomarkers would allow for the development of toxicant-specific tests (e.g., polycyclic aromatic hydrocarbon–DNA adducts) and the detection of subclinical changes that might have significant health implications but which now go unnoticed by current measures. New biomarkers of semen quality are advantageous in that they can both describe male reproductive capacity and indicate toxic effects independent of the female partner's reproductive health. New tests could more accurately measure sperm function, fertilization potential, and the transmission of an intact male genome. Genetic testing may provide valuable tools for researchers and clinicians. For example, the sperm chromatin stability assay and FISH are used to assess genetic structure after exposure to a potential toxicant. Recently, single nucleotide polymorphisms have been used in the assessment of gene–environment interactions.

Another key element of future research is the development of a standardized panel of relevant biomarkers. Individual biomarkers are often used in isolation to test for one specific exposure or type of outcome. However, a panel of biomarkers may, in combination, provide a more powerful lens through which to examine male reproductive capacity and evaluate toxicity. Therefore, researchers need to systematically examine correlation among existing biomarkers and develop standardized panels of complementary biomarkers. With the current tools it is possible to paint a more detailed picture of the relationship between environmental exposures and male reproductive health than has been previously understood. When prospective studies are designed with this approach in mind, epidemiologic models can provide data on the complex kinds of exposure patterns that undoubtedly have an impact on male reproductive health.

Foster interdisciplinary research. Wildlife populations. The discoveries made by ecologists of reproductive abnormalities in wildlife populations have, in the past, alerted medical scientists to the possibility of correlated adverse health outcomes in humans (*108*). There are major differences in the endocrine system across species, so wildlife species must be studied first without extrapolation to humans in mind. However, information about wildlife can be used to guide studies in laboratory animal models, the results of which can be used to guide human research and policy development. The link between ecologic and biomedical research scientists needs to be strengthened so that the adverse health effects of environmental toxicants can be identified and studied quickly, with the goal of prevention. To that end, funding should be earmarked for collaborative multidisciplinary research projects that will bring specialized diverse researchers together to focus on common goals. In addition to establishing collaborative research, funding should be invested to improve wildlife surveillance. This will benefit the field of wildlife biology as well as, ultimately, characterization of wildlife sentinels for human health.

Experimental models. Multigenerational studies with experimental animals, particularly continuous breeding studies, should be used to develop paradigms for human studies. Although animal research is limited in its correlation to human health outcomes, it is a critical component of gaining a better understanding of male reproductive health. Animal studies are beneficial because researchers can control the route and dose of exposure to the suspected toxicant as well as mixtures of toxicants, researchers can look at a wide variety of specific end points, the studies have fewer confounding factors than

in human populations, and laboratory animals are genetically homogenous. Animal research thus allows for the determination of a toxicity threshold that can then be extrapolated to humans.

It is not enough to know that a substance is toxic to the male reproductive system. Scientists must also examine the mechanisms of toxicity. Mechanistic understanding facilitates making predictions about the potential toxicity of individual compounds or complex mixtures, enhances our understanding of the windows of vulnerability in the development of the male reproductive system, and contributes to the development of improved screening tests (including *in vitro* tests). Animal research is an important means of determining the mechanisms of toxicity, and research must proceed in this area. Furthermore, animal data can be used to help formulate hypotheses and design epidemiologic studies in humans, and in turn, human studies can inform toxicology experiments.

Epidemiology. The evidence generated from studies of wildlife and experimental models should guide the development of prospective epidemiologic studies that focus on specific outcomes and measure specific toxicants. Epidemiologists should aim to study populations that are consistent geographically and over time, so that they may avoid confounding factors which can dilute observed associations. Appropriate prospective studies are needed to address geographic variability while taking into consideration or controlling other important variables. These include methods of selecting the study population and methods of semen analysis; seasonal variation; ethnic variation; and measurable environmental factors.

One of the barriers to research in male reproductive health is the perceived or actual difficulty in obtaining semen samples. Epidemiologists should aim to increase response rates in their studies through individualized contact with the study subjects, limited financial incentives, and convenient collection methods.

Existing surveillance systems may be valuable data sources for occupational reproductive health research. Exposure registries for workers exposed to known reproductive toxicants such as heavy metals, pesticides, and radiation, for example, could be used to develop epidemiologic cohorts. Ongoing examination of available data will clarify the extent to which existing surveillance systems can be used.

Standardized birth information forms would greatly aid epidemiologic research in the area of male reproductive health. There is a need to convene a working group to *a*) develop standardized data collection to ensure that equivalent data on potential parental exposures or risk factors are obtained worldwide and *b*) more reliably evaluate environmental exposures or other risk factors during critical periods of development. The development and implementation of uniform computerized records will allow for a more precise understanding of routinely collected data in the future. The systems used in Scandinavian countries can serve as a model for this kind of standardization (*109*). It is also important to increase the type of information collected on birth certificates so that these vital statistics may, in the future, provide the basis for useful retrospective cohort studies.

At present, recommendations for data collection at birth in the United States are made nationally but states implement their own systems. Therefore, there is a clear need for national standards. A working group of experts should be convened to generate computerized pre-, peri-, and postnatal forms for clinicians to use. Although there are drawbacks to computer dependence including limited computer access in some settings, an enormous volume of data management, and the complexity of quality assurance and control, the benefits of standardized data collection outweigh the limitations.

Recognize the importance of standardized laboratory methods and sample archiving. Scientists need to be able to compare data obtained through laboratory testing. However, many of the laboratory methods for assessing male reproductive health are not standardized, which means not only that different laboratories will generate different results using the same sample but also that the results of the same sample within one laboratory may be different when analyzed at different times. Centralization of laboratories is not necessary but standardization of methodologies is necessary. Quality control initiatives involving research and clinical laboratories are necessary to ensure reliability of data. It is critical that the international research community develop standards by which laboratory testing can become more accurate and more precise. This need for standardization applies to exposure as well as outcomes assessment. This will allow for a better characterization of international comparisons or trends in male reproductive health. These programs would encompass tissue archives, including pathologic specimens from tumors, blood banks, and semen banks. Research should be developed that will incorporate the use of archived tissue to provide as broad a picture of male reproductive health as possible. It is important to continue to archive tissue and develop strategies to maximize multiuse research in the future.

Summary

From changes observed in wildlife, to increasing rates of testicular cancer, to the debate regarding trends in sperm counts, there has been increasing concern that hazardous substances in the environment adversely affect male reproductive health. Our ability to fully address the impact of hazardous substances has been hampered by limitations on sources of relevant data and appropriate research methodologies. The recommendations summarized in this paper should serve as a framework for future studies to improve our knowledge in this area. By better defining the problems, learning about the mechanisms responsible for adverse effects, and developing panels of relevant biomarkers, we will make progress toward preventing future adverse effects on male reproductive health.

References and Notes

1. Tas S, Lauwerys R, Lison D. Occupational hazards for the male reproductive system. Crit Rev Toxicol 26(3):261–307 (1996).
2. NIOSH. The National Occupational Research Agenda. NIOSH 96-115. Cincinnati, OH:National Institute for Occupational Safety and Health, 1996.
3. Ekbom A, Akre O. Increasing incidence of testicular cancer—birth cohort effects. APMIS 106:225–231 (1998).
4. Cryptorchidism: an apparent substantial increase since 1960. John Radcliffe Hospital Cryptorchidism Study Group. Br Med J (Clin Res Ed) 29:1401–1404 (1986).
5. Guillette LJ Jr, Brock JW, Rooney AA, Woodward AR. Serum concentrations of various environmental contaminants and their relationship to sex steroid concentrations and phallus size in juvenile American alligators. Arch Environ Contam Toxicol 36(4):447–455 (1999).
6. National Research Council. Biologic Markers in Reproductive Toxicology. Washington, DC:National Academy Press, 1989.
7. Harrison PT, Holmes P, Humfrey CD. Reproductive health in humans and wildlife: are adverse trends associated with environmental chemical exposure? Sci Total Environ 205:97–106 (1997).
8. Fox GA. Practical causal inference for ecoepidemiologists. J Toxicol Environ Health 33:359–373 (1991).
9. Guillette LJ Jr, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ Health Perspect 102:680–688 (1994).
10. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. Persistent DDT metabolite *p,p′*-DDE is a potent androgen receptor antagonist. Nature 375:581–585 (1995).
11. Facemire CF, Gross TS, Guillette LJ Jr. Reproductive impairment in the Florida panther: nature or nurture? Environ Health Perspect 103(suppl 4):79–86 (1995).
12. Ankley G, Mihaich E, Stahl R, Tillitt D, Colborn T, McMaster S, Miller R, Bantle J, Campbell P, Denslow N, et al. Overview of a workshop on screening methods for detecting potential (anti-) estrogenic/androgenic chemicals in wildlife. Environ Toxicol Chem 17:68–87 (1998).
13. Campbell PM, Hutchinson TH. Wildlife and endocrine disruptors: requirements for hazard identification. Environ Toxicol Chem 17:127–135 (1998).
14. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. Br Med J 305:609–613 (1992).
15. Colborn T, Dumanoski D, Myers JP. Our Stolen Future. New York:Dutton Press, 1996.
16. Irvine DS. Falling sperm quality [Letter]. Br Med J 309:476 (1994).
17. James WH. Secular trend in reported sperm count. Andrologia 12:381–388 (1980).
18. Swan SH, Elkin EP, Fenster L. Have sperm densities

declined? A reanalysis of global trend data. Environ Health Perspect 105:1228–1232 (1997).
19. Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guilette LJ Jr, Jégou B, Jensen TK, Jouannet P, Keiding N, et al. Male reproductive health and environmental xenoestrogens. Environ Health Perspect 104(suppl 4):741–803 (1996).
20. Henderson BE, Benton B, Cosgrove M, Baptista J, Aldrich J, Townsend D, Hart W, Mack TM. Urogenital tract abnormalities in sons of women treated with diethylstilbestrol. Pediatrics 58:505–507 (1976).
21. Gill WB, Schumacher GFB, Bibbo M. Pathological semen and anatomical abnormalities of the genital tract in human male subjects exposed to diethylstilbestrol *in utero*. J Urol 117:477–480 (1977).
22. Arai Y, Mori T, Suzuki Y, Bern HA. Long-term effects of perinatal exposure to sex steroids and diethylstilbestrol on the reproductive system of male mammals. Int Rev Cytol 84:235–268 (1983).
23. Brown-Grant K, Fink G, Greig F, Murray MAF. Altered sexual development in male rats after oestrogen administration during the neonatal period. J Reprod Fertil 44:25–42 (1975).
24. Newbold RR, Bullock BC, McLachlan JA. Adenocarcinoma of the rete testis. Diethylstilbestrol-induced lesions of the mouse rete testis. Am J Pathol 125:625–628 (1986).
25. McLachlan JA. Rodent models for perinatal exposure to diethylstilbestrol and their relation to human disease in the male. In: Developmental Effects of Diethylstilbestrol (DES) in Pregnancy (Herbst AL, Bern HA, eds). New York:Thieme-Stratton, 1981;148–157.
26. Newbold RR, McLachlan JA. Diethylstilbestrol associated defects in murine genital tract development. In: Estrogens in the Environment. II: Influences on Development (McLachlan JA, ed). New York:Elsevier, 1985;288–318.
27. Wingo PA, Tong T, Bolden S. Cancer statistics, 1995. CA Cancer J Clin 45:8–30 (1995).
28. Bosl GJ, Motzer RJ. Testicular germ-cell cancer. N Engl J Med 337:242–253 (1997).
29. Skakkebaek NE, Rajpert-De Meyts E, Jørgensen N, Carlsen E, Petersen PM, Giwercman A, Andersen A-G, Jensen TK, Andersson A-M, Müller J. Germ cell cancer and disorders of spermatogenesis: an environmental connection? APMIS 106:3–12 (1998).
30. Adami H-O, Bergström R, Möhner M, Zatonski W, Storm H, Ekbom A, Tretli S, Teppo L, Ziegler H, Rahu M, et al. Testicular cancer in nine northern European countries. Int J Cancer 59:33–38 (1994).
31. Bergström R, Adami H-O, Möhner M, Zatonski W, Storm H, Ekbom A, Tretli S, Teppo L, Akre O, Hakulinen T. Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon. J Natl Cancer Inst 88:727–733 (1996).
32. Levi F, Te V-C, La Vecchia C. Testicular cancer trends in the Canton of Vaud, Switzerland, 1974–1987. Br J Cancer 62:871–873 (1990).
33. Pearce N, Sheppard RA, Howard K, Fraser J, Lilley BM. Time trends and occupational differences in cancer of the testis in New Zealand. Cancer 59:1677–1682 (1987).
34. Thompson IM, Optenberg S, Byers R, Dove M. Increased incidence of testicular cancer in active duty members of the Department of Defense. Urology 53:806–807 (1999).
35. Magoha GA. Testicular cancer in Nigerians. East Afr Med J 72:554–556 (1995).
36. Stat bite: U.S. incidence of testicular cancer [News]. J Natl Cancer Inst 91(21):1803 (1999).
37. Davies JM. Testicular cancer in England and Wales; some epidemiological aspects. Lancet 1(8226):928–932 (1981).
38. Westergaard T, Andersen PK, Pederson JB, Frisch M, Olsen JH, Melbye M. Testicular cancer risk and maternal parity: a population-based cohort study. Br J Cancer 77:1180–1185 (1998).
39. Moller H, Skakkebaek NE. Testicular cancer and cryptorchidism in relation to prenatal factors: case-control studies in Denmark. Cancer Causes Control 8:904–912 (1997).
40. Akre O, Ekbom A, Hsieh CC, Trichopoulos D, Adami HO. Testicular nonseminoma and seminoma in relation to prenatal characteristics. J Natl Cancer Inst 88:883–889 (1996).
41. Moller H, Skakkebaek NE. Risks of testicular cancer and cryptorchidism in relation to socio-economic status and related factors: case-control studies in Denmark. Int J Cancer 66:287–293 (1996).
42. Mills PK. Correlation analysis of pesticide use data and cancer incidence rates in California counties. Arch Environ Health 53:410–413 (1998).
43. Hardell L, Nasman A, Ohlson CG, Fredrikson M. Case-control study on risk factors for testicular cancer. Int J Oncol 13:1299–1303 (1998).
44. Foley S, Middleton S, Stitson D, Mahoney M. The incidence of testicular cancer in Royal Air Force personnel. Br J Urol 76:495–496 (1995).
45. Hardell L, Ohlson CG, Fredrikson M. Occupational exposure to polyvinyl chloride as a risk factor for testicular cancer evaluated in a case-control study. Int J Cancer 73:828–830 (1997).
46. Ramani P, Yeung C, Habeebu S. Testicular intratubular germ cell neoplasia in children and adolescents with intersex. Am J Surg Pathol 17:1124–1132 (1993).
47. Rajpert-De Meyts E, Jorgensen N, Brondum-Nielsen K, Muller J, Skakkebaek NE. Developmental arrest of germ cells in the pathogenesis of germ cell neoplasia. APMIS 106:196–206 (1998).
48. Moller H, Skakkebaek NE. Risk of testicular cancer in subfertile men: case-control study. Br Med J 318:559–562 (1999).
49. Moller H. Trends in sex-ratio, testicular cancer and male reproductive hazards: are they connected? APMIS 106:232–239 (1998).
50. Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 29:1392–1395 (1993).
51. Congenital malformations worldwide: a report from the International Clearinghouse for Birth Defects Monitoring Systems. Oxford:Elsevier, 1991.
52. Paulozzi LJ. International trends in rates of hypospadias and cryptorchidism. Environ Health Perspect 107:297–302 (1999).
53. Auger J, Kunstmann JM, Czyglik F, Jouannet P. Decline in sperm quality among fertile men in Paris during the past 20 years. N Engl J Med 332:281–285 (1995).
54. Bostofte E, Serup J, Rebbe H. Has the fertility of Danish men declined through the years in terms of semen quality? A comparison of semen qualities between 1952 and 1972. Int J Fertil 28:91–95 (1983).
55. Bendvold E. Semen quality in Norwegian men over a 20-year period. Int J Fertil 34:401–404 (1989).
56. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Declining semen quality and increasing incidence of testicular cancer: is there a common cause? Environ Health Perspect 103(suppl 7):137–139 (1995).
57. Fisch H, Ikeguchi EF, Goluboff ET. Worldwide variations in sperm counts. Urology 48:909–911 (1996).
58. Fisch H, Goluboff ET, Olson JH, Feldshuh J, Broder SJ, Barad DH. Semen analyses in 1,283 men from the United States over a 25-year period: no decline in quality. Fertil Steril 65:1009–1014 (1996).
59. Rasmussen PE, Erb K, Westergaard LG, Laursen SB. No evidence for decreasing semen quality in four birth cohorts of 1,055 Danish men born between 1950 and 1970. Fertil Steril 68:1059–1064 (1997).
60. Fisch H, Andrews H, Hendricks J, Goluboff ET, Olson JH, Olsson CA. The relationship of sperm counts to birth rates: a population based study. Urology 157:840–844 (1997).
61. Claudio L, Bearer CF, Wallinga D. Assessment of the U.S. Environmental Protection Agency methods for identification of hazards to developing organisms. Part I: the reproduction and fertility testing guidelines. Am J Ind Med 35(6):543–553 (1999).
62. Chapin RE, Sloane RA, Haseman JK. The relationships among reproductive endpoints in Swiss mice, using the reproductive assessment by continuous breeding database. Fundam Appl Toxicol 38:129–142 (1997).
63. Wyrobeck AJ, Schrader SM, Perreault SD, Fenster L, Huszar G, Katz DF, Osorio AM, Sublet V, Evenson D. Assessment of reproductive disorders and birth defects in communities near hazardous chemical sites. III: Guidelines for field studies of male reproductive disorders. Reprod Toxicol 11:243–259 (1997).
64. Golden AL, Moline JM, Bar-Chama N. Male reproduction and environmental and occupational exposures: a review of epidemiological methods. Salud Publica de Mexico 41(suppl 2):S93–S105 (1999).
65. Whorton D, Krauss RM, Marshall S, Milby TH. Infertility in male pesticide workers. Lancet 2:1259–1261 (1977).
66. Whorton D, Foliart D. DBCP: eleven years later. Reprod Toxicol 2:155–161 (1988).
67. Schrader SM. Male reproductive toxicity. In: Handbook of Human Toxicology (Massaro EJ, ed). Boca Raton, FL:CRC Press, 1997:962–980.
68. Schrader SM, Turner TW, Ratcliffe JM. The effects of ethylene dibromide on semen quality: a comparison of short-term and chronic exposure. Reprod Toxicol 2:191–198 (1988).
69. Bonde JP, Hjollund H, Kolstad H, Abell A, Larsen B. Environmental semen studies: is infertility increased by a decline in sperm count? [Abstract]. Presented at the International Symposium on Environment, Lifestyle and Fertility, 7–10 December 1997, Aarhus, Denmark.
70. Hatch M, Marcus M. Occupational exposures and reproduction. In: Reproductive and Perinatal Epidemiology (Kiely M, ed). Boca Raton, FL:CRC Press, 1993;131–142.
71. Selevan SG. Epidemiology. In: Occupational and Environmental Reproductive Hazards: A Guide for Clinicians (Paul M, ed). Baltimore, MD:Williams & Wilkins, 1993;100–110.
72. Schrader SM, Turner TW, Breitenstein MJ, Simon SD. Longitudinal study of semen quality of unexposed workers. I: Study overview. Reprod Toxicol 2:183–190 (1998).
73. Schenker MB, Samuels SJ, Perkins C, Lewis EL, Katz DF, Overstreet JW. Prospective surveillance of semen quality in the workplace. J Occup Med 30:336–344 (1988).
74. Larsen SB, Abell A, Bonde JP. Selection bias in occupational sperm studies. Am J Epidemiol 147:681–685 (1998).
75. Bar-Chama N, Lamb DJ. Evaluation of sperm function. What is available in the modern andrology laboratory? Urol Clin North Am 21:433–446 (1994).
76. Schrader SM, Turner TW, Simon SD. Longitudinal study of semen quality of unexposed workers. Sperm motility characteristics. J Androl 12:126–131 (1991).
77. Royster MO, Lobdell DT, Mendola P, Perreault SD, Selevan SA, Rothmann SA, Robbins WA. Evaluation of a container for collection and shipment of semen with potential uses in populations-based, clinical and occupational settings. J Androl 21:478–484 (2000).
78. Bigelow PL, Jarrell J, Young MR, Keefe TJ, Love EJ. Association of semen quality and occupational factors: comparison of case-control analysis and analysis of continuous variables. Fertil Steril 69:11–18 (1998).
79. Levine R. Methods for detecting occupational causes of male infertility. Scand J Work Environ Health 9:371–376 (1983).
80. World Health Organization. WHO laboratory manual for the examination of human sperm and semen-cervical mucus interaction, 4th ed. Cambridge, NY:Cambridge University Press, 1999.
81. Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe JP, van Zyl JA, Smith K. Sperm morphologic features as a prognostic factor in *in vitro* fertilization. Fertil Steril 46:1118–1130 (1986).
82. Lipschultz LI. The debate continues—the continuing debate over the possible decline in semen quality. Fertil Steril 65:909–911 (1996).
83. Martin RH, Ernst S, Rademaker A, Barclay L, Ko E, Summers N. Chromosomal abnormalities in sperm from testicular cancer patients before and after chemotherapy. Hum Genet 99:214–218 (1997).
84. Bonde JP, Giwercman A, Ernst E. Identifying environmental risk to male reproductive function by occupational sperm studies: logistics and design options. Occup Environ Med 53:511–519 (1996).
85. Bonde JP, Joffe M, Danscher G, Apostoli P, Bisanti L, Giwercman A, Kolstad H, Thonneau P, Roeleveld N, Vanhoorne M. Objectives, designs and populations of the European Asclepios study on occupational hazards to male reproductive capability. Scand J Work Environ Health 25(suppl 1):49–61 (1999).
86. Schrader SM, Platek SM, Zaneveld IJD, Perez-Palaez M, Jeyendra RS. Sperm viability: a comparison of analytical methods. Andrologia 18:530–538 (1986).
87. Schrader SM, Kesner JS. Male reproductive toxicology. In: Occupational and Environmental Reproductive Hazards. A Guide for Clinicians (Paul M, ed). Baltimore:Williams & Wilkins, 1993;3–17.
88. Santen RJ, Bardin CW. Episodic luteinizing hormone secretion in man: pulse analysis, clinical interpretation, physiologic mechanisms. J Clin Invest 52:2616–2628 (1973).
89. Sokol RZ. Endocrine evaluations in the assessment of male reproductive hazards. Reprod Toxicol 2:217–222 (1988).

90. Schrader SM, Turner TW, Breitenstein MJ, Simon SD. Measuring male reproductive hormones for occupational field studies. J Occup Med 35:574–576 (1993).
91. Apostoli P, Romeo L, Peroni E, Ferioli A, Ferrari S, Pasini F, Aprili F. Steroid hormone sulphation in lead workers. Br J Ind Med 46:204–208 (1996).
92. Halvorson LM, DeCherney AH. Inhibin, activin, and follistatin in reproductive medicine. Fertil Steril 65:459–469 (1996).
93. Jensen TK, Andersson AM, Hjollund NH, Scheike T, Kolstad H, Giwercman A, Henriksen TB, Ernst E, Bonde JP, Olsen J, et al. Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. J Clin Endocrinol Metab 82:4059–4063 (1997).
94. Moosani N, Pattinson HA, Carter MD, Cox DM, Rademaker AW, Martin RH. Chromosomal analysis of sperm from men with idiopathic infertility using sperm karyotyping and fluorescence in situ hybridization. Fertil Steril 64:811–817 (1995).
95. Jacobs PA. The chromosome complement of human gametes. In: Oxford Review of Reproductive Biology (Milligan SR, ed). New York:Oxford University Press, 1992;47–72.
96. Martin R, Redemaker A. The effect of age on the frequency of sperm chromosomal abnormalities in normal men. Am J Hum Genet 41:484–492 (1987).
97. Martin RH, Spriggs E, Rademaker AW. Multicolor fluorescence in situ hybridization analysis of aneuploidy and diploidy frequencies in 225,846 sperm from 10 normal men. Biol Reprod 54:394–398 (1996).
98. Spriggs EL, Rademaker AW, Martin RH. Aneuploidy in human sperm: the use of multicolor FISH to test various theories of nondisjunction. Am J Hum Genet 58:356–362 (1996).
99. Kinakin B, Rademaker A, Martin R. Paternal age effect of YY aneuploidy in human sperm, as assessed by fluorescence in situ hybridization. Cytogenet Cell Genet 78:116–119 (1997).
100. Robbins WA, Vine MF, Truany KY, Everson RB. Use of fluorescence in situ hybridization (FISH) to assess effects of smoking, caffeine, and alcohol and aneuploidy load in sperm of healthy men. Environ Mol Mutagen 30:175–183 (1997).
101. Rubes J, Lowe X, Moore D II, Perreault S, Slott V, Evenson D, Selevan SG, Wyrobek AJ. Smoking cigarettes is associated with increased sperm disomy in teenage men. Fertil Steril 70:715–723 (1998).
102. Petrini J, Alter C, Andrews H, Damus K. Men have babies too: paternal factors and pregnancy outcomes [Abstract]. Presented at the International Conference on Hazardous Substances and Male Reproductive Health, 14–15 May 1998, New York, New York.
103. Robbins WA, Rubes J, Selevan SG, Perreault SD. Air pollution and sperm aneuploidy in healthy young men. Environ Epidemiol Toxicol 1:125–131 (1999).
104. U.S. Environmental Protection Agency. Health Effect Test Guidelines. Reproduction and Fertility Effects. OPPTS 870.3800. Washington, DC:U.S. Government Printing Office, 1998.
105. Organisation for Economic Co-operation and Development. OECD's Guidelines for the Testing of Chemicals, Section 4—Health Effects. TG Nos. 414, 415, 416. Available: http://www.oecd.org/ehs/test/health.htm [cited 14 June 2000].
106. U.S. Environmental Protection Agency Office of Pollution Prevention and Toxics. Chemical Hazard Data Availability Study. Available: http://www.epa.gov/opptintr/chemtest.htm [cited 14 June 2000].
107. Pope AM, Rall DP, eds. Environmental Medicine: Integrating a Missing Element into Medical Education. Washington, DC:National Academy Press, 1995.
108. American Academy of Pediatrics. Handbook of Pediatric Environmental Health (Etzel RA, Balk SJ, eds). Elk Grove Village, IL:American Academy of Pediatrics, 1999.
109. van der Schalie WH, Gardner HS Jr, Bantle JA, De Rosa CT, Finch RA, Reif JS, Reuter RH, Backer LC, Burger J, Folmar LC, et al. Animals as sentinels of human health hazards of environmental chemicals. Environ Health Perspect 107:309–315 (1999).
110. Lunde AS, Lundeborg S, Lettenstrom GS, Thygesen L, Huebner J. The person-number systems of Sweden, Norway, Denmark, and Israel. Vital Health Stat 2:1–59 (1980).