Science linking environmental contaminant exposures with fertility and reproductive health impacts in the adult male

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In the field of reproductive environmental health there remain many unanswered questions regarding the impact of the environment on male reproductive health. Suggested needs include studies that target populations with high exposure to chemicals, including phthalates and bisphenol A. We also need to identify susceptibility factors and critical exposure windows (life stages) that may increase a man's risk of infertility. Finally, we need to develop methods to better study mixtures of chemicals and develop methods to assess clinical reproductive outcomes of human exposure to the ever-growing list of chemicals. (Fertil Steril® 2008;89:e59–65. ©2008 by American Society for Reproductive Medicine.)

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Documentation of chemicals as male reproductive toxicants dates back to Roman times (1). In the past 2 decades, numerous animal and clinical studies have provided evidence that a variety of chemicals can disrupt the hypothalamic–pituitary–testicular axis by acting as hormonal antagonists or agonists and/or by disrupting the biochemical processes regulating hormone secretion and sperm function. The ultimate clinical outcome of exposure to reproductive toxicants is hypogonadism and/or infertility (2, 3). Reproductive toxicants can be categorized into heavy metals, agricultural chemicals, and industrial chemicals.

Historically, clinical observation has identified a particular chemical to be a male reproductive toxicant. Animal studies, in turn, lead to an understanding of the toxicity of the chemical and ultimately serve as the basis of clinical studies. Until recently, the risk assessment framework has served as the recommended scientific approach to characterizing reproductive toxicants. Risk assessment is a four-step paradigm: [1] hazard identification, [2] dose–response assessment, [3] exposure assessment, and [4] risk characterization (4). Biologic endpoints studied include measurement of circulating levels of LH, FSH, testosterone, estradiol, and other hormones as indicated; semen analysis and sperm function testing; and biochemical and genetic markers. This experimental approach is perhaps best exemplified by studies designed to assess the reproductive toxicity of the heavy metal lead acetate, and the agricultural chemical dibromochloropropane (DBCP).

Lead was identified as an abortifacient and a cause of male infertility and impotence (hazard identification) during the days of the Roman Empire (1). However, it was the pioneering study of Lancranjan and colleagues (5) that focused attention on the role that chemicals might play in male factor infertility. These investigators studied reproductive outcomes in men who worked on the production line and compared them to men working in the office of a battery plant in Eastern Europe. They reported a dose-related suppression of spermatogenesis, normal or decreased serum T, and inappropriately normal urinary gonadotropins in the face of low testosterone levels in men with higher blood lead levels.

A number of investigators turned to animal studies to study dose–response and exposure assessment of lead exposure. Adverse reproductive effects appear to be dose-related, reversible, and age related, with pubertal animals the most sensitive (6). Mechanistic studies suggested that lead exposure disrupts all levels of the reproductive axis (7–9). Follow-up clinical studies are less definitive than the animal studies, but support the evidence that toxicity occurs at all levels of the reproductive axis, with some studies concluding that the primary site of toxicity is the central nervous system, with others concluding that the gonad is the most sensitive organ (6, 10, 11). Recent evidence suggests that lead interferes with the ability of spermatozoa to undergo the acrosome reaction, thus leading to infertility (12). Lead exposure may induce chromosomal abnormalities (13). Genetic variability in response to lead exposure is suggested by the finding that ion channel polymorphisms may cause differential sensitivities to lead exposure both in the animal model and clinically (14).

Although the regulation of lead-containing gasoline has dramatically decreased lead exposure, workers continue to be exposed if they work in manufacturing plants where lead is a component of the final product (15). Studies evaluating other heavy metals suggest that cadmium, mercury, and boron may disrupt male reproduction (11, 16).

DBCP, a nematocide that was widely used in agriculture in the United States and Europe until 1977, serves as another

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key example of the risk assessment approach to the study of a reproductive toxicant. Although preliminary animal studies identified DBCP as a testicular toxicant, it was not until the 1970s that it was identified clinically as a cause of male infertility and sterility (17–21) The initial studies reported by Whorton et al. (17) documented a direct relationship between sperm count and duration of DBCP exposure in DBCP production workers in Northern California. Animal and human studies confirmed that DBCP is a testicular toxin (22). Recovery of spermatogenesis and fertility by exposed men is variable following removal of the exposure (20, 21). Although DBCP use is now prohibited in the United States, well water continues to be contaminated in some states, and DBCP use is still allowed in a number of countries.

Other agricultural chemicals implicated as male reproductive toxicants include epichlorhydrin, DDT, ethylene dibromide, kepone, vinclozalin, and the dioxins (23, 24).

Although the risk assessment approach provides a great deal of information about dose response and mechanisms of toxicity, human exposure to most substances identified as reproductive toxicants is not at toxic dose levels. Therefore, we turn to epidemiologic evidence to adequately characterize a toxicant's risk.

Compared with the evidence on lead and DBCP, the evidence on the potential impact on male reproductive function of other environmental chemicals is quite limited. Despite this, recently published epidemiologic studies provide suggestive evidence that exposure to some environmental chemicals may have adverse effects on male reproductive function. The remaining sections describe epidemiologic evidence on environmental challenges to male fertility assessed by conventional semen parameters (25) and newer biologic markers such as sperm DNA damage (26, 27).

Epidemiologic studies are generally designed to target specific study populations such as high-exposure subpopulations with a wide range of exposure variability, as well as subgroups with increased susceptibility to the contaminant of interest. Epidemiologic studies also target chemicals with large potential public health impact, which is dependent upon their risk and the proportion of the population exposed to the chemical.

We artificially divide the following discussion according to studies that focus on high-exposure subpopulations and those that focus on chemicals with widespread human exposure. As expected, there is overlap among these study types, and this distinction is meant purely for organizational purposes. In addition to a general description of epidemiologic designs, a set of specific more detailed examples will be used to motivate the discussion. This discussion is not meant to be exhaustive, but instead, will focus on some of the recent informative epidemiologic studies.

HIGH-EXPOSURE STUDIES

Populations with high exposure to chemicals relevant to male fertility include, among others: [1] individuals occupationally exposed, [2] members of communities with high ambient exposures, [3] individuals undergoing medical procedures, and [4] those accidentally exposed to high levels. Phthalates are one class of chemicals with potential adverse effects on male fertility. Workers may be exposed to high levels of phthalates at factories manufacturing polyvinyl chloride products (28) or cable manufacturers (29). Levels within these occupational settings may be 10 to 100 times, or higher, than background general population exposure.

Among farmers and workers formulating pesticides, high exposure may occur. There is some evidence of associations of organophosphate pesticides with decreased semen quality (30). Another contaminant of interest is air pollution. Members of communities with high exposure include those living in neighborhoods near power plants or in communities with meteorologic and geographic characteristics that contribute to high air pollution levels. Data on semen quality and air pollution comes from studies within the United States (31) and Czech Republic (32).

Another set of studies on individuals with high exposure includes those with exposure from medical treatments. Currently, these studies are limited to confirming the suspected high exposure to phthalates among neonatal intensive care unit infants (33–35) and have largely not explored associations with later development or reproductive health (36).

The final category of high-exposure studies includes individuals with high exposure because of accidents such as that which occurred in Seveso Italy in 1976, when a chemical manufacturing plant exploded (37). Another example occurred in Japan and Taiwan, where large groups of individuals were exposed to high levels of polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans because of accidental contamination of rice oil during processing (38). The PCBs leaked from the heat exchangers into the oil and consumers were unaware. Several studies have explored the association between high exposure to PCBs and semen quality among these populations (39). In addition, there are several studies on semen quality and exposure to general population PCB levels (39).

POLYCHLORINATED BIPHENYLS

Polychlorinated biphenyls are a class of synthetic, persistent, lipophilic, halogenated aromatic compounds that were widely used in industrial and consumer products for decades before their production was banned in the United States in the late 1970s. Polychlorinated biphenyls were used in cutting oils, lubricants, and as electrical insulators. As a result of their extensive use and persistence, PCBs remain ubiquitous environmental contaminants. They are distributed worldwide, and have been measured in air, water, aquatic, and marine sediments, fish, and wildlife (40). Furthermore, they are biologically concentrated and stored in human adipose tissue. The general population is exposed primarily through ingestion of contaminated foods (e.g., fish, meat, and dairy products), as PCBs bioaccumulate up the food chain. As a result of their

persistence and ubiquity, measurable levels of serum PCBs are found in the majority of the US general population (41).

Semen Quality and High PCB Exposure

Guo and colleagues (42) studied the relationship between semen quality and prenatal exposure to PCBs and PCDFs after the poisoning episode in Taiwan in 1979, in which PCB contaminated rice oil was ingested. Twelve men with prenatal exposure to contaminated rice oil and 23 healthy unexposed subjects of comparable age provided a semen sample. The unexposed men had no unusual chemical exposure, and were recruited from a local high school. In the exposed men the proportion of sperm with abnormal morphology was increased and the percentage of motile sperm and rapidly motile sperm were reduced. In addition, sperm from exposed men had reduced hamster oocyte penetration compared with unexposed men. This small study provided the opportunity to explore high prenatal exposure to PCBs and PCDFs.

In a second study on men from the Taiwan PCB poisoning episode, Hsu and coworkers (43) studied the relationship between semen quality and levels of PCBs among men that consumed contaminated rice oil some 20 years earlier. They identified 40 exposed men and 28 unexposed men of similar age. Exposed men had a higher percentage of sperm with abnormal morphology and a higher oligozoospermia rate. The ability of sperm to penetrate the hamster oocyte was reduced in exposed men.

Semen Quality and Environmental PCB Exposure

In one of the early studies on environmental exposure to PCBs and semen quality, Bush and coworkers (44) reported that seminal plasma levels of the PCB congeners 153, 138, and 118 were inversely related to sperm motility but only among semen samples with a sperm count less than 20 million/mL. In a more recent study in The Netherlands, Dallinga and colleagues (45) studied men that were partners in couples visiting an infertility treatment center. Based on progressive motile sperm concentration, they identified a group of men with good semen quality ($n = 31$) and a group of men with very poor semen quality ($n = 34$). Contrary to expectations, the sum of PCBs in seminal plasma of men with good semen quality was higher than among men with poor semen quality. However, within the group of men with good semen quality, there were inverse associations between serum levels of sum of PCB metabolites and sperm count and progressive motile sperm concentration. There were also inverse nonsignificant corresponding associations in the men with poor semen quality. Because associations with semen quality were found for PCB metabolites and not the parent PCBs, these results suggested that the PCB metabolites were the biologically active compounds.

In Sweden, Richthoff and coworkers (46) studied 305 young men 18 to 21 years of age undergoing a conscript examination for military service. There were significant inverse associations between PCB 153, a biomarker of exposure to total PCBs, and percent motile sperm. There were no associations between PCB 153 and sperm concentration or total sperm count. In India, Rozati and coworkers (47) studied infertile men and controls. They reported a negative correlation between seminal plasma PCB levels and total progressive motility and a positive correlation with percentage of single-stranded DNA in sperm. No correlations were found between PCBs and sperm count, rapid progressive motility, or normal morphology. The investigators reported results for total PCBs and not for individual congeners. Potential confounders were considered in the Methods section, but no adjustments were made in the analysis.

Rignell-Hydbom et al. (48) studied 195 Swedish fishermen from the east and west coasts. The highest PCB 153 quintile had decreased sperm motility compared with men in the lowest quintile. There were no consistent associations of PCB 153 with sperm concentration. In the United States, Hauser and colleagues (49) studied 212 male partners of subfertile couples visiting an infertility clinic. There were significant dose–response relationships between PCB 138 and below World Health Organization (WHO) reference sperm motility and sperm morphology. Associations between semen parameters and PCB 153 were not consistent.

The data on the relationship between PCBs and semen quality support an inverse association of PCBs with reduced semen quality, specifically reduced sperm motility (39). The associations found were generally consistent across studies performed in different countries (India, The Netherlands, Taiwan, Sweden, and the United States) that used different methods to measure semen quality and PCBs. Furthermore, associations were consistently found despite a range of PCB levels, that is, there did not appear to be a threshold. Although the data across studies generally support a relationship between PCBs and poor semen quality, there are possible alternative explanations. One potential alternative explanation is that PCBs are a surrogate for exposure to other environmental factors that may predict semen quality. Although this is possible, there is currently no evidence identifying potential alternative exposures. Another explanation is that there may be confounding of the associations by some currently unrecognized or unmeasured confounders. Although possible, this is also unlikely because the more recent studies considered important potential confounders and the results were consistent across studies. In conclusion, although PCBs are no longer used, this data, along with ongoing human exposure, albeit at lower levels than several decades ago, raise concerns regarding altered human fertility because of adverse affects on semen quality.

CHEMICALS WITH WIDESPREAD EXPOSURE

Because the study subdivisions are artificial, some of the chemicals described above in the high-exposure subpopulation section are discussed here. Specifically, there are several epidemiologic studies on exposure to background levels of pesticides (50–52) and PCBs (44–49) with reduced semen quality. Additional contaminants with widespread low-level

exposure include metals such as lead and cadmium for which there is evidence of associations with semen quality at low-level exposure levels (11). Ambient air pollution is also included in this category of widespread exposure because most individuals in industrialized countries are exposed to air pollutants.

Additional classes of chemicals with widespread exposure that are of current interest include those used in consumer products, such as phthalates, bisphenol A, and parabens. As far as we are aware, human data on risks of bisphenol A and parabens on semen quality are not available. The animal data show associations of BPA with poor semen quality (53, 54).

PHTHALATES

Low-level background exposure to phthalates will be used as a specific example of a chemical with widespread general population exposure that may impact male fertility. High molecular weight phthalates, for example, di-(2-ethylhexyl) phthalate (DEHP), are primarily used as plasticizers in the manufacture of flexible vinyl plastic, which in turn, is used in consumer products, flooring and wall coverings, food contact applications, and medical devices (55, 56). Manufacturers use low molecular weight phthalates (e.g., diethyl phthalate [DEP] and dibutyl phthalate [DBP]) in personal care products (e.g., perfumes, lotions, cosmetics), as solvents and plasticizers for cellulose acetate, and in making lacquers, varnishes, and coatings, including those used to provide timed releases in some pharmaceuticals (55, 57, 58).

As a result of the ubiquitous use of phthalates in personal care and consumer products, human exposure is widespread. Exposure through ingestion, inhalation, and dermal contact are considered important routes of exposure for the general population (56–60). Parenteral exposure from medical devices and products containing phthalates are important sources of high exposure to phthalates, primarily DEHP (33, 56). Upon exposure, phthalates are rapidly metabolized and excreted in urine and feces (56–58). The most common biomonitoring approach for investigating human exposure to phthalates is the measurement of urinary concentrations of phthalate metabolites.

Compared with the laboratory animal data on the reproductive toxicity of phthalates, the human data is limited. In an early study on phthalates and semen quality, Murature and coworkers (61) measured DBP concentrations in the cellular fractions of ejaculates from 21 university students and found an inverse relationship with sperm concentration. The study was small, and did not adjust for potential confounders. In another small study, conducted in India, Rozati and coworkers (47) studied 21 infertile men with poor semen quality and 32 control men with normal semen parameters. The concentration of the sum of several phthalate diesters in seminal plasma was inversely correlated with sperm morphology but not correlated with ejaculate volume, sperm concentration, or motility. In this study, as in the Murature study, the measurement of phthalate diesters raises concern with sample contamination from the ubiquitous presence of the diester in the environment.

More recently, a larger study using urinary levels of phthalate metabolites was conducted by Hauser and colleagues (62). Study subjects consisted of male partners of subfertile couples who presented to an infertility clinic in Massachusetts. There were dose–response relationships (after adjusting for age, abstinence time, and smoking status) between monobutyl phthalate (MBP, the hydrolytic metabolite of DBP), and below WHO (25) reference value sperm motility and sperm concentration. There was also a dose–response relationship between monobenzyl phthalate (MBzP, the primary hydrolytic metabolite of BBzP) and below WHO reference value sperm concentration.

In a recently published study from Sweden, Jonsson and colleagues (63) recruited young Swedish men at the time of their medical conscript examination. In contrast to the US study, in the Swedish study there were no relationships of MBP or MBzP with any of the semen parameters. MEHP was also not associated with any of the semen parameters. Men in the highest quartile for MEP had fewer motile sperm and more immotile sperm than men in the lowest MEP quartile. Contrary to their hypothesis, phthalic acid was associated with improved function as measured by more motile sperm and fewer immotile sperm. Phthalic acid is a nonspecific marker of phthalate exposure, formed as the result of the hydrolysis of any of the phthalates measured.

Although the Swedish study had some similarities to the US study (they were both cross-sectional studies in which a single urine and semen sample were collected), there were many important differences. One of the primary differences was in the age of the study population and the method of recruitment. The Swedish study population consisted of young men (median age 18 years, range 18–21 years) undergoing a medical examination before military service. Because approximately 95% of young men in Sweden undergo the conscript examination, these young men reflected the general population of young Swedish males. In contrast, in the US study, the median age of the men recruited from an infertility clinic was 35.5 years and ranged from 22 to 54 years. None of the men from the infertility clinic were 21 years of age or younger. The differences across studies in the ages and source of the men may account for some of the differences in results between studies. For instance, it is unclear whether men presenting to an infertility clinic are more "susceptible" to reproductive toxicants, including phthalates, than men from the general population. Furthermore, it is also unclear whether middle-aged men, compared with young men, are more "susceptible" to reproductive toxicants because of an age-related response to the toxicant.

In summary, the epidemiologic data on the relationship between semen quality and phthalate exposure remains limited and inconsistent (39). Although the two recent studies by Hauser et al. (62) and Jonsson et al. (63) had some similarities, important differences existed. Additional studies

are critically needed to help elucidate possible explanations for differences across studies, and most importantly to address whether phthalate exposure alter semen quality.

In summary, the epidemiologic evidence on environmental chemicals and semen quality is limited. However, for several classes of chemicals or environmental contaminants, such as phthalates, PCBs, pesticides, and air pollution, the evidence is intriguing, and further studies are warranted to confirm the preliminary suggestive results. For those chemicals with very limited or no human data, epidemiologic studies need to be designed and implemented because of the large potential public health impact.

Several critical research directions, using the same categories as above for epidemiologic approaches, will be described. Although we define critical directions within each category, we recognize that there is overlap. High-exposure population studies on health risks of early life exposure to phthalates among neonates in intensive care units is critically needed. These babies are at risk of the confirmed high exposures they receive at critical sensitive windows of life when testicular development and immature mechanisms of metabolism may render them highly susceptible. Several classes of medications contain phthalates, leading to high exposure among pregnant women as well as young boys prescribed these medications. Therefore, studies on the implications for the male fetus, especially later life reproductive function, are warranted. We need to recognize that although these studies span decades of an individual's life, this should not deter epidemiologists from studying high exposure subpopulations because they are at highest risk. Several of the phthalate containing medications (such as Asacol) are prescribed for many thousands of individuals with inflammatory bowel disease, both pregnant women and young boys.

Another critical research direction is for studies on bisphenol A, widely used to manufacture polycarbonate plastics and for the lining of metal cans (64). Because of the consistent animal data, epidemiologic studies on BPA are needed, especially in high-exposure subpopulations.

Critical research directions for chemicals with widespread low-dose exposure overlap with those described above for chemicals with high exposure. Apart from identifying high BPA exposure subpopulations, we need to conduct studies among members of the general population with low-level environmental BPA exposure. Another critical research need is to design studies that collect data on and allow for analysis of risks from mixtures of chemicals. Individuals are exposed to most if not all of the chemicals and metals described above, and developing methods to understand fertility impacts of mixtures is desperately needed.

Another evolving issue is the substitution of the organophosphate pesticides with pyrethroids. Because of their widespread use and limited human data (52), more studies are needed. Finally, general background exposure to metals such as mercury and cadmium warrants further investigation into their risks to male reproductive function.

In conclusion, we would like to note that the studies and study designs described above need to incorporate markers of susceptibility to these chemicals and metals. Susceptibility may be increased by the timing or life stage at which exposure occurs, for instance, neonatal or peripubertal periods. In addition, genetic susceptibility may result from differences incurred because of genetic polymorphisms in enzymes metabolizing or activating these compounds. With the current revolution in genomics and molecular biology, these techniques must be incorporated into the next generation of epidemiologic studies on male reproductive health.

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