Research | Workgroup Report

An Occupational Reproductive Research Agenda for the Third Millennium

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There is a significant public health concern about the potential effects of occupational exposure to toxic substances on reproductive outcomes. Several toxicants with reported reproductive and developmental effects are still in regular commercial or therapeutic use and thus present potential exposure to workers. Examples of these include heavy metals, organic solvents, pesticides and herbicides, and sterilants, anesthetic gases, and anticancer drugs used in health care. Many other substances are suspected of producing reproductive or developmental toxicity but lack sufficient data. Progress has been limited in identifying hazards and quantifying their potencies and in separating the contribution of these hazards from other etiologic factors. Identifying the causative agents, mechanisms by which they act, and any potential target populations, present the opportunity to intervene and protect the reproductive health of workers. The pace of laboratory studies to identify hazards and to underpin the biologic plausibility of effects in humans has not matched the pace at which new chemicals are introduced into commerce. Though many research challenges exist today, recent technologic and methodologic advances have been made that allow researchers to overcome some of these obstacles. The objective of this article is to recommend future directions in occupational reproductive health research. By bridging interdisciplinary gaps, the scientific community can work together to improve health and reduce adverse outcomes. *Key words:* communication, environmental exposure, occupational exposure, reproduction, research design, risk factors. *Environ Health Perspect* 111:584–592 (2003). doi:10.1289/ehp.5548 available via *http://dx.doi.org/* [Online 28 October 2002]

There is a significant public health concern about the potential effects of occupational exposure to toxic substances on reproductive outcomes. Many toxicants with reported reproductive and developmental effects are still in regular commercial or therapeutic use and thus present potential exposure to workers. Examples of these include heavy metals (lead, cadmium), organic solvents (glycol ethers, toluene, vinyl chloride), pesticides and herbicides (ethylene dibromide), and sterilants, anesthetic gases, and anticancer drugs used in health care. Many other substances are suspected of producing reproductive or developmental toxicity.

Absence of sufficient toxicologic and epidemiologic data lends a degree of uncertainty to the estimated magnitude of risk that a specific toxicant presents. Against this backdrop, government public health agencies are charged with assuring the safety and health of wage earners in the workplace. Unfortunately, the majority of chemical toxicants regulated by such agencies are not evaluated for reproductive or developmental toxicity. In a 1991 U.S. General Accounting Office (U.S. GAO) report prepared for the U.S. Senate, 30 chemicals considered to be reproductive and developmental toxicants were reviewed. The study found that information on reproductive or developmental toxicities was not available or used in setting exposure limits for the majority of these chemicals (U.S. GAO 1991).

The report concluded that public health "protection against reproductive and developmental toxicity offered to the public by current regulation is uncertain at best" (U.S. GAO 1991).

It is estimated that 10–20% of recognized pregnancies end in spontaneous abortion or stillbirth (Hatasaka 1994) and as many are lost before recognition of pregnancy (Wilcox et al. 1988; Zinaman et al. 1996). In addition, 10–15% of couples are unable to conceive after 1 year of unprotected intercourse (Chandra and Stephen 1998; Rudolph and Forest 1990). Approximately 3% of all live births have major malformations [Centers for Disease Control and Prevention (CDC) 1995; Holmes 1997; International Clearinghouse for Birth Defects Monitoring Systems 1991; National Center for Health Statistics 1998]. It is estimated that 3% of these major malformations are strictly due to toxicant exposure (Kimmel 1997; Oakley 1986), 28% can be attributed to genetic causes, and approximately 23% are attributable to multifactorial causes, which are complex interactions between genes and environmental factors (Holmes 1997). The cause is unknown in more than 40% (Holmes 1997; Wilson 1973). What is known, however, is that identifying the causative agents, mechanisms by which they act, and any potential target populations will present the opportunity to intervene and better protect the reproductive health of the public.

Progress has been limited in identifying hazards and quantifying their potencies and in separating the contribution of these hazards from other etiologic factors. The pace of laboratory studies to identify hazards and to underpin the biologic plausibility of effects in humans has not matched the pace at which new chemicals are introduced into commerce. In addition, the number of workers of reproductive age potentially exposed to occupational chemical and physical agents has grown. The percent of children born to working mothers has risen steadily from 31% in 1976 to 59% in 1998 (U.S. Census Bureau 2000a), and approximately 65% of employed women and men are of reproductive age (U.S. Census Bureau 2000b), thus providing an increased probability of occupational reproductive exposures.

Occupations with exposure to potential reproductive toxicants include veterinary, dental, and medical health care workers (Cordier et al. 1991; Fedoruk 1996; Giacoia 1992; Khattak et al. 1999; McDiarmid 1988; McDonald 1988; Paul and Himmelstein 1988; Saurel-Cubizolles et al. 1985; Welch 1986); lab technicians (Giacoia 1992; McDonald 1988; Paul and Himmelstein 1988); agricultural workers (McDonald 1988; Office of Technology Assessment Task Force 1985; Olshan et al. 1989; Vaughan et al. 1984); workers in construction (McDonald 1988); automobile mechanics and auto body shop workers (Daniell and Vaughan 1988; Giacoia 1992; Paul and Himmelstein 1988); firefighters (Giacoia 1992); chemical industry workers (Office of Technology Assessment Task Force 1985); iron workers (Olshan et al. 1989); janitorial and cleaning persons (McDonald 1988; Olshan et al. 1989); dry cleaning and laundry persons (Giacoia 1992); and persons working in different trades including metal fitters, electronic technicians, machinists and other metal tradesmen, and

building and vehicles tradesmen (Ford et al. 1994). Several occupational exposures have been reported in the literature as being associated with adverse reproductive outcomes, including pesticides, solvents, and pharmaceuticals (Giacoia 1992; Khattak et al. 1999; Mattison and Thomford 1989; McDonald 1988; Office of Technology Assessment Task Force 1985; Paul and Himmelstein 1988; Rachootin and Olsen 1983; Welch 1986). However, studies that detail the relationship between specific exposures and adverse reproductive outcomes are much less common.

In 1993 Marcus et al. published an article outlining a reproductive hazards research agenda for the 1990s, and many of those issues still apply today (Marcus et al. 1993). As stated in that article, research on reproductive outcomes is complicated because of the intricate biology of reproduction, the multiple targets involved (male, female, offspring), the uncertainties in extrapolating from model species to humans, and the problems involved in accurately characterizing exposure-related outcomes in epidemiologic investigations. The public health relevance of the study of reproductive outcomes is not limited to couples attempting to conceive. Because the endocrine system is involved in many physiologic processes, impaired reproduction may be a marker for higher risk of reproductive cancers (e.g., breast, ovarian, testicular), cardiovascular disease, osteoporosis, and age-related cognitive decline. Though reproductive research is complicated and challenging, recent technologic and methodologic advances have been made that allow scientists to overcome some of these obstacles.

In 1996 the National Institute for Occupational Safety and Health (NIOSH) and its partners implemented the National Occupational Research Agenda (NORA) in which approximately 500 organizations and individuals outside NIOSH provided input for the development of an agenda to improve occupational safety and health. As one of the 21 research priority areas of NORA, members of the Fertility and Pregnancy Abnormalities Team have collaborated on this article to develop research priorities. Thus, the objective of this article is to recommend future directions in occupational reproductive health research, with the purpose of reducing the incidence of adverse reproductive health outcomes. This can be accomplished with an interdisciplinary research program that identifies reproductive hazards, their mechanisms of toxicant action, and target populations.

Reproductive outcomes research. Reproductive and developmental toxicity refers to the continuum of adverse effects that may befall an exposed child, parent, or pregnant woman and her offspring exposed *in utero*. Outcomes of reproductive performance

include infertility or subfertility and pregnancy loss, and structural malformations, growth retardation, or functional deficits in the developing organism. Exposure to toxicants during gestation, postnatal development, or later in life may affect gametogenesis, ovulatory function, sexual function, timing of puberty and menopause, fertilization, implantation, or any aspect of the developmental process. Effects may be directly on the reproductive system or embryo or indirectly on the endocrine system that regulates reproduction.

Investigative studies of animal test species can elucidate the mechanisms of action of a toxicant. Studies for testing model species can also include standard safety evaluations that comprehensively assess the potential for a chemical to affect the reproductive process. Such studies evaluate the ultimate outcomes of reproduction and development; that is, they are apical in nature but provide little information about the pathways by which adverse effects occur. Numerous end points have been added to these study protocols over the past decade to make them more sensitive and to include measurements that are more immediately interpretable in the context of fertility end points measured in humans [Claudio et al. 1999; Clegg et al. 2001; U.S. Environmental Protection Agency (U.S. EPA) 1998a]. These include measurements of sperm production/sperm number, sperm motility and morphology, duration and regulation of estrous cycle, and time of onset of puberty. Additional end points have been added to enhance the ability of these assays to determine the potential for chemicals to affect estrogen, androgen, and thyroid hormone function.

Identifying Hazards

A discrepancy exists between the number of chemicals in commerce (approximately 84,000) and the number that have been evaluated in model species for reproductive toxicity potential (4,000) (U.S. EPA 1998b). It is not feasible to allocate additional resources to test the 80,000 or so untested chemicals through traditional testing protocols, particularly given that about 2,000 new chemicals are introduced into commerce each year (U.S. EPA 1998b). Instead, new, more rapid methods are needed to screen large numbers of chemicals and to identify those that are potential reproductive hazards. In the near term, top priorities will be to develop the most promising alternative models and to test their ability to appropriately classify the toxicity of sets of known toxicants and nontoxicants. Successful alternative tests can then be applied to the large list of chemicals of unknown activity. The result would be a prioritized list for more comprehensive, traditional toxicity testing in model species, accompanied by field studies for those compounds for which human exposure is high or widespread.

High-throughput assays. High-throughput assays evaluate the effect of a test substance on a single biologic process using an automated manner that allows thousands or tens of thousands of compounds to be tested in a short time at a reasonable cost. Robotics and genetic engineering make it possible to produce large quantities of receptors or genetically engineered cells for use in these assays.

Knowledge about mechanisms of toxicity is often central to the strategy of high-throughput assays. For example, cells are being developed that are bioengineered to express human hormone receptors for estrogens and androgens. These cells can be used for high-throughput chemical screening for steroid hormone receptor affinity or the potential to act as endocrine disruptors. Both isoforms of recombinant human estrogen receptor and human androgen receptor are commercially available for this purpose. The U.S. EPA plans to use high-throughput assays for estrogens and androgens to screen their initial list of 15,000 chemicals in the Toxic Substances Control Act inventory and prioritize them for further evaluation using more elaborate screens and tests (U.S. EPA 1998b). Based on the same principles, other batteries of high-throughput assays are available to screen for activity against various receptors and cytochrome P-450 enzyme isoforms. The availability and application of these assays will undoubtedly expand as we understand more about the relevance of each protein in toxicologic processes.

Another approach for moderate or high-throughput screening is to use genetically sensitized embryos of simple organisms to screen for effects on signal transduction. Signal transduction is the process by which receptors send information of ligand binding to the rest of the cell and elicit a response. Compared with the plethora of receptors that have been identified, there are relatively few types of signal transduction; 17 pathways have been identified, and the pace of discovery suggests that there are few left to be revealed [National Research Council (NRC) 1989]. Therefore, it is likely that toxicants that affect different receptors may use the same signal transduction pathway. If so, then analysis of signal transduction pathways may prove to be a tractable approach to high-throughput screening that does not require setting up thousands of receptor-based assays or pinpointing an exact mechanism of toxicity.

Structure–activity prediction. Methods for predicting activity from structure continue to be developed and refined. Computer programs use available empirical information about the toxicity of existing compounds and their chemical characteristics to predict whether a new compound will have similar toxicity. These programs have not performed well in the area of reproductive and developmental toxicity, probably because reproductive

processes are complex and effects may be elicited through multiple modes and mechanisms. As science progresses and we learn more about mechanisms of toxicity at the molecular level, however, structure–activity computer programs will become more exact and predictive. The best examples are the programs that are being developed and refined for estrogen receptor binding (Tong et al. 1997).

Integration of human studies and tests of model species. Though 4,000 chemicals have been tested in model species, few chemicals have been adequately evaluated for reproductive effects in humans (Tables 1 and 2 contain a partial list of known human developmental and adult toxicants). Because the interpretation of studies of model species is often not straightforward and because field studies are labor and resource intensive, a systematic approach is needed to select and prioritize chemicals for epidemiologic studies. Moorman et al. (2000) recently proposed a process for selecting chemicals for human field studies. In this process, information gained from model species testing conducted by the National Toxicology Program (NTP) was reviewed for significant adverse reproductive effects and potency of the toxicants. The evaluative process then combined this information with human exposure information available in public databases to arrive at a list of high-priority candidates for studies in humans. Requests for Applications (RFAs) have been used to encourage human studies of some of these high-priority substances. In 2000, NIOSH, the U.S. EPA, the National Institute of Environmental Health Sciences (NIEHS), and the National Cancer Institute cosponsored RFA "Endocrine Disruptors: Epidemiologic Approaches" to encourage investigations of developmental effects of potential endocrine disruptors. In 2001, NIOSH sponsored RFA "Occupational Exposure to Putative Reproductive/Developmental Toxicants in Humans" to stimulate studies of potential reproductive/developmental effects of the NTP-tested chemicals.

Latent effects. Functional deficits are adverse developmental outcomes that do not have obvious structural correlates. The detection of functional deficits has long been recognized as a potential manifestation of developmental toxicity, but functional deficits have not been examined routinely in hazard assessments of model species. Heightened awareness of (and concern for) these effects have led to the modification of model species studies for testing toxicity to include assessments of the latent effects on the developing nervous, immune, and reproductive systems. These studies will provide important information on the range of potential outcomes that may occur after developmental exposure to a toxicant. Detection of such effects in humans

will be a challenge because the separation in time of chemical exposure and observation of an effect will hinder the identification of causal factors. Birth defect registries cannot account for such effects, and recall of early exposures by adolescents or adults manifesting effects is likely to be limited. It will be important to conduct studies that record early exposures and include follow-up of functional competence in adulthood. Such studies have not yet been conducted. However, the CDC, the National Institutes of Health (NIH), and the U.S. EPA are collaborating to propose a large cohort study of infants followed into adulthood to determine whether prenatal/perinatal exposures are associated with long-term effects (Children's Health Act of 2000).

New biomarkers for humans and model species. In 1977, men exposed to dibromochloropropane (DBCP), a pesticide that is now banned in the United States, were found to be azoospermic and oligospermic (Whorton et al. 1977). Currently, a variety of biomarkers are used to assess the potential adverse reproductive effects due to toxic chemical exposures. Bioindicators of sperm production and quality (semen volume, sperm concentration, sperm motility, sperm morphology) are routinely evaluated in ejaculated semen samples in men and in suspensions of epididymal sperm from test species (epididymal sperm reserves, sperm motility, sperm morphology) (Moline et al. 2000; U.S. EPA 1998a). During the past decade, computer-assisted methods developed to improve and automate the evaluation of sperm motion and morphology have been added to the battery of routine sperm measures, and guidance for their use and interpretation has been made available through a number of workshops (Chapin et al. 1992; e.g. ESHRE 1998; ILSI 1999; Schrader et al. 1991; Seed et al. 1996). Furthermore, baseline data on the relationship between various semen or epididymal sperm measures and fertility have emerged from a number of large studies designed to address this question (Bonde et al. 1998; Chapin et al. 1997; Zinaman et al. 2000). Thus, these measures are widely accepted biomarkers of adverse reproductive effects that are suitable for application in both human and model species studies. Serum hormone measures can also be determined in humans and test species. Inhibin B has been proposed as an indicator of testicular function and a possible surrogate for sperm measures (Anderson and Sharpe 2000).

The recognition that sperm functional tests are also desirable has led to development of various new tests that have only recently been applied to toxicology. Biomarkers of the genetic integrity of sperm are designed to identify risks for paternally mediated developmental effects. Sperm proteins are being tested as biomarkers of fertility to detect specific

deficits in sperm function (as opposed to decreased sperm output). Although details of such tests are beyond the scope of this article, Table 3 provides a list of new tests and references regarding methodologic details and examples of use. Further research is needed to make these tests more practical and more cost effective and to determine their ultimate utility for hazard identification and elucidation of modes and mechanisms of toxicant action.

In females, biomarkers of effect include the timing of onset of menarche, the pattern of menstrual cyclicity, time to pregnancy, and loss of fertility, as well as the measurement of circulating steroid hormone (estrogen and

Table 1. Partial list of known human developmental toxicants.[a,b]

Physical
  Ionizing radiation (atomic weapons, radioiodine, therapeutic X ray)
  Hyperthermia
Biologic
  Cytomegalovirus
  Herpes simplex virus I and II
  Parvovirus B-19
  Rubella virus
  Syphilis
  Toxoplasmosis
  Varicella virus
  Venezuelan equine encephalitis virus
Chemical
  Alcoholism
  Aminopterin and methylaminopterin
  Androgenic and antiandrogenic hormones
  Busulfan
  Captopril
  Chlorinated biphenyls
  Cigarette smoking
  Cocaine
  Coumarin anticoagulants
  Cyclophosphamide
  Diethylstilbestrol
  Diphenylhydantoin
  Enalapril
  Etretinate
  Lead
  Lithium
  Mercury, organic
  Methimazole
  Misoprostol
  Penicillamine
  13-*cis* Retinoic acid
  Tetracyclines
  Thalidomide
  Valproic acid

[a]Developmental toxicants are agents that cause structural malformations, pregnancy loss, growth retardation, or functional deficits. [b]Adapted from Shepard 1998.

Table 2. Human adult reproductive toxicants.

Alcoholism
Antineoplastic agents
Ethylene glycol methyl ether
Carbon disulfide
Cigarette smoking
Dibromochloropropane
Ethylene glycol monoethyl ether
Hyperthermia
Lead

progesterone) and pituitary hormone [follicle-stimulating hormone (FSH), luteinizing hormone, prolactin] levels. Biologic markers used to assess effects of toxic chemicals during pregnancy include fetal viability, measurement of circulating and/or urinary placental hormone levels (estriol, progesterone, human chorionic gonadotropin, human placental lactogen), and the occurrence of developmental abnormalities, especially in hormone-sensitive tissues (NRC 1989). Because first-trimester pregnancy loss can be an imprecise tool, studies incorporating cytogenic methods can offer the advantage of documenting aneuploid and normal diploid karyotypes in exposed populations (Marchetti et al. 1997; Silverman et al. 1985).

Biologic monitoring allows for evaluation of hormone levels during menstrual cycles (Kesner et al. 1999; Lipson and Ellison 1996; Waller et al. 1998). An important consideration in the overall validity of serum hormone measurements has been pulsatility, or within-subject temporal fluctuations (Carandente et al. 1989; McShane et al. 1996). Since the 1980s, investigators have been developing indices of menstrual cycle function from hormones or hormone metabolites excreted in first-morning urine. Hormonal profiles derived from blood samples have traditionally been used to characterize clinically recognized conditions such as polycystic ovarian syndrome, anovulation, and luteal-phase deficiency. Hormones and hormone metabolite levels in urine generally parallel circulating serum profiles of these hormones but would not be expected to be identical. Urine hormone levels are unlikely to reflect pulsatile secretion patterns possibly found in serum, and individual variation in steroid metabolism and routes of excretion can also create differences (Lasley et al. 1993). Nonetheless, investigators have characterized multiple indices of menstrual function, including urine hormone levels and hormone ratios, in groups of normal women. Several of these markers are associated with conditions including anovulation (Kassam et al. 1996), reduced fertility, and the probability of conception (Baird et al. 1999). Menstrual function studies of women working in the semiconductor industry (Gold et al. 1995a), with jet fuel (Reutman et al. 2002), or as flight attendants (Whelan et al. 2002) have been conducted or are in progress. Because many of these markers vary with stage of life, analyses should correct for age. As more information becomes available on the use of these markers in women exposed to potential reproductive toxicants, they are likely to become important descriptors of women's reproductive health in occupational studies. Urinary hormone assays for additional endocrine end points such as inhibin A and B and leptin may also be useful.

Surveillance of human populations. Improvement and expansion of public health databases that can be tracked on a state- and nationwide scale are crucial to identifying causes of chronic disease and disability. Historically, environmental causes of developmental and reproductive defects were first recognized by clinicians. For example, rubella was shown to cause congenital cataracts, and thalidomide was found to be teratogenic. In both of these examples, and in others, the association was discovered by an epidemic of rare cases that occurred over a short time frame. However, occupationally and environmentally related diseases may not be as easily identified if they produce a less severe, more common outcome, making them more difficult to detect against a low but predictable background of occurrence. Systematic collection of data makes identification of such relationships more feasible and cost effective.

Birth defect tracking in the United States is handled on a state-by-state basis, and not all states have a system in place (PEW 2000).

In 1997 the CDC established birth defects centers in seven states: Arkansas, California, Iowa, Massachusetts, New Jersey, New York, and Texas. The CDC National Center for Birth Defects and Developmental Disabilities (NCBDDD) in Atlanta participates as the eighth center. Each center is a collaboration among state health departments, local hospitals, universities, and the state chapter of the March of Dimes Birth Defects Foundation. A main activity of the centers is to participate in the National Birth Defects Prevention Study, a case–control study of major structural birth defects (Yoon et al. 2001).

Traditionally, data available in the few existing birth defect surveillance systems rarely included information on potential occupational or environmental exposures. There are two issues regarding existing birth defect surveillance with respect to occupation: *a*) How can existing systems be better used for epidemiologic research, including research on occupational exposures? *b*) Can parental occupation and industry data be reliably incorporated into existing surveillance systems?

Establishment of a national cancer death registry has been a useful tool in testing hypotheses regarding the relationship between environmental exposures and cancer occurrence. This registry allows researchers to evaluate the distribution of disease by age, race, sex, geography, and other related factors. Incorporation of occupational exposure information into existing surveillance systems requires an investment of additional resources. To correctly address the issue of linking the timing of exposure to a critical susceptibility period, questionnaires should include information on employment during pregnancy. Currently, NIOSH is working with the CDC NCBDDD to develop an approach to exposure assessment of parental occupational data that were collected as part of the National Birth Defects Prevention Study. Analyses will not be able to account for congenital anomalies in fetuses that were miscarried or electively terminated and therefore not represented in birth registries. However, registries of birth defects and of other adverse developmental and reproductive outcomes, though difficult and expensive to do, could generate new etiologic hypotheses and improve the feasibility of the research.

Estimating occupational exposure. Establishing that a significant number of workers or members of the general population are or will be exposed to a potential reproductive toxicant is central to priority setting. NIOSH's National Occupational Hazard Survey and National Occupational Exposure Survey conducted in 1972–1974 and 1981–1983, respectively, has been used extensively to identify substances of common exposure (NIOSH 1978, 1988). These surveys are

Table 3. Emerging biomarkers of male reproductive effects.

Target	Bioassay	Function assessed	Selected references and reviews
Sperm DNA	SCSA TUNEL COMET CMA3 staining DNA adducts	Chromatin damage DNA damage	Evenson 1999 Perreault et al. 2000
Sperm chromosomes	FISH	Aneuploidy, breakage, translocation	Robbins et al. 1999 Hassold 1998 Sloter et al. 2000
Fertilizing ability	SP-22 protein Sperm antigens Ubiquitin		Welch et al. 1998 Diekman and Herr 1997 Sutovsky et al. 2001
Sperm maturity	Cytoplasmic droplets Heat shock protein A2		Huszar et al. 2000
Sperm count surrogate	Inhibin B	Endocrine feedback of spermatogenesis	Anderson and Sharpe 2000

Abbreviations: CMA3 staining, chromomycin A3 staining; COMET, single-cell gel electrophoresis assay; FISH, fluorescence *in situ* hybridization; SCSA, sperm chromatin structure assay; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin end-labeling.

the only comprehensive assessments of general industry where the number of workers potentially exposed to chemical agents has been estimated. However, these databases are outdated and of limited use because they indicate only potential exposure. NIOSH is currently planning a new hazard surveillance activity that will target industry sectors on a rolling basis, beginning with the health care sector. Public health researchers will continue to require updated exposure surveys to keep up with the changing workplace exposures and monitor new exposures that may be potential reproductive toxicants. New technologies such as geographic information systems (GIS) allow mapping of industries and specific chemical exposures. Use of GIS to identify geographic areas with high volume of use of suspect chemicals might be an effective method of identifying populations with greater potential occupational and environmental exposures.

In the absence of birth defects surveillance systems, the coding of parent's occupation and industry (O/I) could be collected on vital statistics records or national surveys of reproductive health. For example, the National Survey of Family Growth and the National Maternal and Infant Health Survey have limited O/I information. Although occupation and industry are part of the National Health and Nutrition Examination Survey (NHANES), this information cannot be linked with each pregnancy time period. Additional survey data could be added to NHANES and its updates, providing an opportunity for epidemiologic studies, but such additions are costly and compete with other priorities. Through the NORA program, NIOSH has made efforts to leverage intramural resources to develop collaborative research in NORA areas. As an example, collaboration between NIOSH and Harvard researchers is under way to obtain exposure and reproductive information in the Nurses' Health Study, a large cohort study of female nurses, by adding a supplemental questionnaire for a subset of the cohort.

Parental occupation is recorded on birth and fetal death certificates in some states. NIOSH has supported efforts to include O/I on birth certificates in about 20 states and to develop software to reduce verbatim O/I information to Bureau of Census codes. The NIOSH Standardized Occupation/Industry Coding System software (SOIC) can be used for birth or death certificates and reduces verbatim O/I input to 1990 Bureau of Census codes with 86–88% success. Cancer registries are an existing data source for childhood cancer incidence. Approximately 45 states are funded to maintain cancer registries, but most do not collect O/I of parents. A few states have begun to add this information. Improvement in existing surveillance by

wider use of O/I data might increase our ability to identify new exposures for study.

Biomonitoring is a valuable tool for estimating occupational exposure. Male and female reproductive information and biomarkers (e.g., age at menopause, FSH) have been incorporated into some portions of the NHANES survey. The National Report on Human Exposure to Environmental Chemicals is a new and ongoing assessment of the U.S. population's exposure to environmental chemicals based on data from CDC's NHANES 99 (CDC 2001). The first edition of the report presents levels of 27 environmental chemicals, including metals (e.g., lead, mercury, uranium), cotinine (a marker of tobacco smoke exposure), organophosphate pesticide metabolites, and phthalate metabolites. Scientists at the CDC and NTP reported results of analysis of human urine for the metabolites of phthalate plasticizers (Blount et al. 2000). This is a significant step forward in assessing the potential human toxicity of a class of chemicals known to be reproductive and developmental toxicants in rodents. Improved methods for analysis of exposure, especially of age and time effects, are likely to impact the characterization of occupational exposure in these studies (Richardson and Wing 1998).

Current research approaches usually consider the action of single, unique toxicants on outcomes of interest, creating yet another challenge to drafting a reproductive hazards agenda. The more common human exposure scenario is to mixtures of toxicants at low concentrations, episodically and over the long term. Attention to cumulative exposure over years of a working lifetime and total aggregate exposure to toxicants from multiple exposure sources, as well as classical considerations of exposure routes, must also be addressed. Methodologic approaches must enlarge and mature to consider the effects and modulation of effects mediated by both exposure to mixtures of toxicants and the complexities of exposure mode at low dose and over prolonged duration.

Epidemiologic studies of occupational exposures. Not all health effects are easily identified through surveillance systems such as registries. Timing and dose of exposure affect the outcome. Adverse pregnancy outcomes such as spontaneous abortion are not routinely reported, and many cognitive defects are identified several years after birth. Reproductive effects such as altered semen quality in men and changes in hormone balance in both men and women require laboratory analyses and would warrant a more extensive surveillance system to identify changes in distribution over person, place, or time. Because of limited registry data, researchers have used a combination of company work histories and validated

reproductive questionnaire information to study reproductive outcomes, including fetal loss, birth defects, reduced fertility (several indices including time to pregnancy), sex ratio, birth weight, and gestational age.

Menstrual function biomonitoring studies can complement pregnancy outcome studies by measuring sensitive hormonal markers of female infertility and subfertility in smaller groups of female workers. As described earlier, methods developed in clinical settings have now been modified for workplace biomonitoring studies to enable women to collect and store series of daily urine and/or saliva samples, often in conjunction with diary information. These studies should lead to better understanding of the responses to exposure.

Male reproductive studies are modest in cost, require relatively few participants, and can indicate the potential dysfunctional impact of a reproductive toxicant with minor changes in the male reproductive profile. Workplace studies of over 50 measures of semen quality, male reproductive hormones, and survey measures of potency and libido have described longitudinal normal semen quality values for working men (Schrader et al. 1998) and identified reproductive hazards (Grajewski et al. 1996, 2000; Ratcliffe et al. 1989; Schrader et al. 1988; Whelan et al. 1996). Though difficult to do, if national cohort studies included semen analyses along with other biologic samples, it would provide important population-based data with which to compare exposed workers.

Opportunities in Mechanistic Research

Understanding mechanisms of action of toxicants is important for a number of reasons, including *a*) supporting the biologic plausibility of an observed association between chemical exposure and adverse outcome; *b*) uncovering common pathways of actions of different agents; *c*) extrapolating across species for risk assessment; *d*) improving the predictability of human morbidity from responses of model species; and *e*) predicting responses to mixed exposures.

Mechanistic studies are not new in toxicology; however, new tools in genomics, proteomics, and bioinformatics present unprecedented opportunities to advance our understanding of toxicant action at a molecular level. Genomic information and the ability to screen most or all of the genome of an increasing number of organisms for changes in gene expression are revolutionizing the way in which biologic effects data are gathered. It is now possible to determine the effects of a toxicant exposure on gene expression of most of the genome of mice and rats. This will allow us to generate testable hypotheses about the mechanism of action of toxicants. It will also

open up the possibility of identifying markers of exposure or effect specific to a particular insult that can be used in field studies. Such markers will aid in determining whether statistical associations between a given exposure and effect have biologic plausibility. For example, a recent study identified selective genes that were expressed *in vitro* in decidualized human endometrial stromal cells in response to progesterone or cyclic adenosine monophosphate (Popovici et al. 2000).

As with any new technology, a number of problems will need to be overcome for the promise of genomics to be realized. The first will be to manage the large volume of information produced by gene expression experiments. Gene chips may contain thousands or tens of thousands of sequences. Experience shows that any perturbation in a biologic system leads to numerous changes in gene expression. An entire field of bioinformatics is being developed to help collect, organize, and manage the data to identify changes related temporally, by dose, or by metabolic pathway. The second challenge will be to separate those changes in gene expression pivotal to the toxic response from those that are more generalized responses to any stimulus. The third challenge will be to quantitatively relate changes in expression of critical genes with toxicity, which is manifested at a more complex level of biologic organization (i.e., the cell, organ, or organism). Real comfort in this genomic approach will come only with experience and the development of a large database. Toward this end, NIEHS recently announced the establishment of the National Center for Toxicogenomics (NCT; Anonymous 2001).

Gene–Environment Interactions

Reproductive toxicants can affect human populations over the total life span, including the *in utero* and perinatal periods, childhood, puberty, and adulthood. Thus, extending research efforts to address stage-specific sensitivity is recommended. Another emerging approach allows the identification of populations at potentially increased risk from toxicant exposure by characterizing genetic polymorphisms of metabolizing enzymes in exposed cohorts. Such methods may identify vulnerable subpopulations on the basis of inherent (genetic) differences in their ability to metabolize a toxicant.

Identifying genes that increase sensitivity to reproductive toxicants. Genetic factors that elevate risk for disease can be grouped into two categories: those for which having a particular allele conveys a high risk for the disease regardless of other (e.g., environmental) influences, and those associated with only small increases in risk of the disease. The latter, termed susceptibility genes, are being identified at an increasing rate. The interaction

of these alleles with environmental agents or other susceptibility alleles ultimately determines whether the disease will be manifested. Much work has been done to understand the role of these genes in cancer etiology [see Caporaso and Rothman (1999) for a review], and some susceptibility genes for reproductive toxicity are now being identified. Identifying these genes will be critical in understanding the role that the environment plays in the 25% of birth defects described as being multifactorial in nature, as well as in many or most of those of unknown etiology. Other reproductive gene–environment interactions are being reported, most recently for an association between low-level occupational benzene exposure and shortened gestational length modified by the presence of two susceptibility genes (Wang et al. 2000). In another study a high rate of oral clefts (cleft lip with or without cleft palate) was reported for offspring of mothers who were heavy cigarette smokers during pregnancy but only if the baby carried a variant allele of the transforming growth factor-alpha gene (Hwang et al. 1995; Shaw et al. 1996). Cigarette smoking combined with the genetic variant increased the risk more than 10-fold compared with the reference group. Rare alleles of the *Msx1* gene, a homeobox gene involved in early embryonic pattern formation, are associated with a slightly increased risk for limb reduction defects. Cigarette smoking during pregnancy doubles the risk of limb defects in infants with the rare alleles; cigarette smoking alone had no effect (Hwang et al. 1998). These examples show the power of genetic analysis in combination with traditional case-control studies in identifying risk factors for abnormal development.

As we learn more about the human genome and about individual polymorphisms that alter susceptibility, it will be possible to design molecular epidemiology studies more routinely to include analysis of putative susceptibility genes. On 1 October 1990, the Human Genome Project (HGP) was initiated through partnership and funding by the NIH and the U.S. Department of Energy. At least 20 international research centers (referred to as the International Human Genome Consortium) collaborated on this public project. The initial goals of this project were to map the entire human genome, develop the technology to accomplish the sequencing of DNA, and characterize the genomes of model laboratory organisms such as yeast, bacteria, worms, flies, and mice, all within a time frame of 15 years. It was the expressed goal of the HGP that all sequence data generated by this effort be freely available and remain in the public domain. Because of steady progress in achieving the initial goals of this project, the aims were expanded to include *a*) complete the sequencing of the entire human genome by

2003, *b*) further improve the current sequencing technology, *c*) identify and map single nucleotide polymorphisms (SNPs) distributed throughout the genome, and *d*) elucidate the function of each gene (by examining the expression pattern of each gene under various experimental conditions), as well as several additional goals (Collins et al. 1998). In 1997 NIEHS presented a plan called the Environmental Genome Project (EGP) that would characterize the variations (SNPs) in selected human genes and relate these differences to variations in susceptibility to environmental chemicals (Kaiser 1997). Although there is potential for overlap in the identification of SNPs between the HGP and EGP, current plans of the EGP are to focus on an estimated 200 genes coding for proteins/enzymes involved in drug metabolism, oxidative stress responses, cell cycle components, and DNA repair mechanisms (Guengerich 1998). In 1998 a biotechnology company, Celera Genomics (the commercial project), announced its plans to sequence the human genome. In February 2001, a draft sequence of the human genome was published by the International Human Genome Consortium (International Human Genome Consortium 2001) and Celera Genomics (Venter et al. 2001). Although this draft sequence is incomplete, it lays the foundation for understanding the genetic basis of disease susceptibility and the individual variability in sensitivity/responsiveness to drugs. Of course, in addition to genomic data, information on epigenetic changes that influence gene expression may be important in understanding susceptibility and adverse response to environmental contaminants (Holliday 1998).

Potential information from genetics to advance epidemiologic studies. One of the goals of the HGP is to identify and map SNPs and to determine how these variations confer susceptibility or resistance to human diseases, whereas the more limited goal of the EGP is to identify SNPs of selected genes that may confer increased sensitivity to environmental toxins. After the DNA sequence variations are identified, they must be correlated with functional changes in the expression and/or activity of protein/enzymes encoded by the genes at the tissue/cellular level. If epidemiologic studies could identify genetic–toxicant interactions by comparing the prevalence of a particular genetic marker (polymorphism) or a group of markers in affected and unaffected populations (Collins et al. 1997), this information could be used to target environmental, behavioral, or medical interventions (Khoury 1997). Ultimately, validation of genetic testing to link a particular genotype with exposure to a specific chemical to the increased prevalence of a particular reproductive disorder would require epidemiologic confirmation (Khoury

and Dorman 1998). Although genetic testing will enhance the sensitivity of epidemiologic studies, this information must be used judiciously to protect individuals from discrimination, as there are ethical issues regarding identification of vulnerable populations in the workplace. Controlling exposures in the workplace remains the best option for preventing occupationally related disease for all workers.

Communication

An essential component of future reproductive studies will be improved communication. Because of the complex mechanisms involved in reproductive research, collaboration across scientific disciplines must be conducted. In addition, notification of research results and recommendations must be communicated to workers and the affected public in a manner that is timely, accessible, and easily understood.

Partnerships across disciplines. In current and future studies, the measurement and characterization of adverse health effects due to reproductive toxicants will depend more on the understanding and collaboration of partners in multiple disciplines. Epidemiologists, toxicologists, molecular biologists, and statisticians have found it essential and ultimately very valuable to collaborate to take advantage of new methodologies. Two key partners, epidemiologists and toxicologists, have begun to work together, although the nature of each of these disciplines has led to operational discomfort along the way (Swan and Lasley 1991).

The motivation to form partnerships across disciplines also derives from the limitations of each of the individual disciplines and the potential to overcome these limitations. For the epidemiologist, biologic monitoring can increase study power, increase the precision of exposure assessment to minimize misclassification, and decrease selection bias. This becomes increasingly important in studying lower levels of exposure, for which the nature of the outcome response will depend on the mechanisms of toxicologic effects (Guidotti 1995). For the toxicologist, well-designed observational human studies offer the generalization to humans often lacking in toxicologic study design (Pershagen 1999).

One example of beneficial multidisciplinary collaboration has been the development of field methods and the analysis of urinary reproductive hormones and hormone metabolites (Swan and Lasley 1991). Study participant collection of small amounts of daily urine for one or more menstrual cycles has been successful in several environmental studies and in occupational groups including semiconductor workers (Gold et al. 1995b) and actively traveling flight attendants (Whelan et al. 2002). Modification of the hormone assays for field use has resulted in an effective biomonitoring metric for ovulatory function, early pregnancy loss, premature menopause, and other important reproductive outcomes. Moving these tests successfully into the field requires that laboratory specifications for stability, preservation, and sample size be met; that the laboratories meet the analysis demands of repeated measures from the relatively large numbers of women in these studies; and that the epidemiologists and laboratory scientists reach consensus on study design elements, including laboratory-based definitions of outcomes and units of observation. This last requirement often results in the unanticipated benefit that both disciplines begin to think about study end points and outcomes in a new way (e.g., a laboratory-based, epidemiologically meaningful definition of "pregnancy" or "early menopause").

Several formal collaborative efforts have been organized. The Center for the Evaluation of Risks to Human Reproduction (CERHR) was established in 1998 by the NTP and NIEHS. The center provides scientific assessments of reproductive health risks associated with human chemical exposures. These assessments are being made available to the public through the CERHR website (CERHR 2002). The NCT, recently established by NIEHS, is another example of a collaborative effort aimed at advancing scientific knowledge of biologic responses for public health use. The NORA Fertility and Pregnancy Abnormalities Team is working with the CERHR and others to develop reproductive research priorities and to increase communication between professionals and the public (NIOSH 2003).

These examples of collaboration should encourage researchers to work together more in the future. Reporting results at shared scientific meetings would also inform our collective understanding, suggest avenues of collaboration, and build efficiently on existing knowledge while avoiding redundancy. Within the government research community, a mechanism is needed to routinely share research agendas and possibly chart collaborations. Presently, this activity is accomplished passively or only through personal communication. There is a role for larger public–private partnerships in both achieving research goals and disseminating results as well. The NIOSH NORA program is one example of such a partnership.

Communicating research findings. Advances in reproductive toxicology and epidemiology lose their public health relevance if the enhanced findings these methods permit do not reach policy makers, employers, and affected persons and help them to make informed decisions. For example, we note that both government agency and industry-sponsored research results may not routinely be published in the peer-reviewed literature. Often, reasons for failing to do this are not as much due to trade secrets as to time and resource constraints of the investigators.

Developing and providing effective communication is a major challenge within the public health and occupational health communities. Effective health communication is a component of intervention effectiveness, another NORA priority area currently framing new NIOSH intramural research projects. Material Safety Data Sheets (MSDSs) are a primary means of communicating hazards to workers. Paul and Kurtz (1994) surveyed Massachusetts MSDSs for products containing two known reproductive toxicants, lead and glycol ethers, and found that over 60% did not mention possible reproductive health effects. Reproductive risk information for workers in clinical settings or participating in health studies has often been absent, poorly written, or unclear. As a result, workers' understanding of reproductive hazards is deficient. Although maternal recall of reproductive history has been found generally to be of good quality (Selevan 1980), a recent survey indicated relatively poor maternal recall of potential workplace reproductive hazards (Bauer et al. 1999).

The message that reproductive health includes both men and women, and that it can be affected by their workplace exposures, needs to reach the workers and their employers. Paul and Kurtz (1994) found that where reproductive hazards were mentioned in MSDSs, they were 18 times more likely to address developmental effects than male reproductive risks. Of 50 patients surveyed who visited an occupational reproductive consulting service, 39 were women with clinically recognized pregnancies. Only 1 man and 10 women contemplating pregnancy were seeking counseling (Frazier and Jones 2000). Generally, NORA Team researchers have found that workers are very interested in reproductive health, and that nontechnical summaries of study findings, as well as personal results, are well received and understood by these groups.

Summary and Recommendations

A primary goal of reproductive research is to reduce the high percentages of adverse outcomes such as infertility, pregnancy loss, and congenital malformations. Although certain limitations exist that are unique to reproductive research, many advances in technology and methodologies have been recently developed that will aid researchers in their efforts to *a*) understand mechanisms by which toxicants exert their effects, *b*) identify populations at risk, and *c*) evaluate reproductive and developmental hazards to improve public health. These tools and resources include

- High-throughput assays
- Structure–activity prediction
- Biomarkers of exposure and effect
- Exposure databases
- GIS
- NIOSH SOIC System
- Genomics
- Proteomics
- Bioinformatics
- HGP and EGP

With the use of these new tools comes a responsibility among researchers to improve existing efforts and reach out into new areas of research. The authors make the following recommendations:

- Prioritization of research needs
  - New toxicology studies should be prioritized based on chemical structure and volume of use.
  - Field studies should be prioritized based on toxicologic studies combined with human exposure information.
- Potential surveillance activities
  - Evaluate occupational exposure data available from existing surveillance systems.
  - Expand additional birth defects surveillance systems to include a greater population in the United States.
  - Add reproductive biologic markers and semen characteristics to national surveys.
- New studies should assess gene–environment interactions and effects of mixtures of chemicals whenever appropriate.
- Research results should be communicated to the policy makers and affected public through widely accessible, nontechnical reports and summaries.
- Improved communication among research disciplines and should be encouraged through
  - Interdisciplinary research protocols
  - Organized collaborative teams
  - Shared scientific meetings/workshops
  - Dissemination of results to wider audiences.

By bridging interdisciplinary gaps, the scientific community can work together to improve health and reduce adverse health outcomes.

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